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# RADIOACTIVE VITAMIN $B_{12}$ ABSORPTION IN THE MEGALOBLASTIC ANAEMIA CAUSED BY ANTICONVULSANT DRUGS[1]

By F. LEES

(From Manchester Royal Infirmary)

ANTICONVULSANT drugs are a well recognized, though uncommon, cause of megaloblastic anaemia. The exact aetiology of this anaemia has been incompletely elucidated, although folic acid has been curative when employed. Phenytoin was implicated in cases described by Badenoch (1954), Chalmers and Boheimer (1954), Hawkins and Meynell (1954), Rhind and Varadi (1954), Webster (1954), Berlyne, Levene, and McGlashan (1955), Ryan and Forshaw (1955), Vaishnava (1955), Barker (1957), Benians and Hunter (1957), Forshaw (1957), Varadi (1957), Hawkins and Meynell (1958), and Stokes and Fortune (1958). Primidone was the offending drug in some of the cases described by Berlyne, Levene, and McGlashan (1955), Fuld and Moorhouse (1956), Girdwood and Lenman (1956), Benians and Hunter (1957), Volterra and Romualdi (1957), and Stokes and Fortune (1958). Barbiturates were implicated in the production of macrocytosis in a series of epileptics studied by Hawkins and Meynell (1958). Amylobarbitone and quinalbarbitone caused megaloblastic anaemia in a patient of Hobson, Selwyn, and Mollin (1956), and phenobarbitone was blamed for this condition by Girdwood and Lenman (1956). The present study concerns the therapeutic effect of vitamin $B_{12}$, the absorption of radioactive vitamin $B_{12}$, and the changes in absorption before and after curative therapy, in cases of megaloblastic anaemia caused by anticonvulsant drugs.

In macrocytosis caused by anticonvulsant drugs, without anaemia, Hawkins and Meynell (1958) measured serum vitamin-$B_{12}$ levels, and found them to be normal. Twenty-two control subjects had levels of from 105 to 385 $\mu\mu$g. per ml. of serum, with a mean level of 215 $\mu\mu$g. per ml. The patients with macrocytosis had a mean level of 175 $\mu\mu$g. per ml. An injection of 100 $\mu$g. of cyanocobalamin was given weekly for two months to six such patients, without effect on the blood picture. Folic-acid absorption was normal, and so was the excretion of parenteral doses of folic acid. Treatment with 20 mg. of folic acid daily by mouth in six patients cured the macrocytosis. Plasma ascorbic-acid levels were rather low, but this was thought not to be significant. There was no evidence of liver disease or of dietary insufficiency. It is generally assumed that macrocytosis due to such drugs is an early stage of megaloblastic anaemia, but the proportion of patients developing the latter condition is not known.

[1] Received October 20, 1960.

Investigations designed to test absorptive and structural alimentary changes have been reported. Fat-balance studies, gastrointestinal barium series, and gastric mucosal biopsies were reported by Badenoch (1954). Hawkins and Meynell (1958) investigated fat balances. Chanarin, Elmes, and Mollin (1958) studied the absorption of $^{131}$I-labelled fat and of vitamin A, glucose tolerance, and barium series. Chalmers and Boheimer (1954) tested fat and glucose absorption. All these tests gave negative or normal results. Rhind and Varadi (1954) found a fat absorption of only 58 per cent. in a three-day test in one patient. Histamine test meals have shown free gastric acid in most cases. Achlorhydria may be coincidental, though it always raises a doubt in diagnosis, and Addisonian pernicious anaemia has to be considered. One of Badenoch's (1954) patients had achlorhydria, but gastric biopsy was normal. Ascorbic-acid deficiency has been described by Kidd and Mollin (1957). Chalmers and Boheimer (1954) found ascorbic-acid unsaturation. Hawkins and Meynell (1958) found rather low levels of plasma ascorbic acid. A similar deficiency was present in some patients of Gydell (1957), and possibly also in those of Newman and Sumner (1957).

Folic-acid tests have not indicated a deficiency, but rather a failure to utilize normal serum levels. The results obtained by Hawkins and Meynell (1958) in studying macrocytosis have already been mentioned. In megaloblastic anaemia due to anticonvulsant drugs Girdwood and Lenman (1956) found normal folic-acid absorption. Chanarin, Elmes, and Mollin (1958) found normal absorption and serum clearance before treatment, and while the patients were taking primidone, as well as during treatment with ascorbic acid and with folic acid. The fact that Christenson, Ultmann, and Roseman's (1957) patient was cured merely by the withdrawal of primidone may be taken to indicate that there was no tissue deficiency of folic acid. Folic acid appears to be curative, and to prevent recurrences of the anaemia, even when the offending drug is continued. Withdrawal of the folic acid may allow a relapse of anaemia. Iron deficiency may be revealed after a response of the megaloblastic anaemia to haematinics.

Data regarding the part played by vitamin $B_{12}$ have been recorded by some authors. It has been mentioned already that in the macrocytosis caused by anticonvulsant drugs Hawkins and Meynell found the average level of vitamin $B_{12}$ per ml. of serum to be 40 $\mu\mu$g. below the average level in control subjects. In the megaloblastic anaemia two patients of Kidd and Mollin (1957) had levels of 50 $\mu\mu$g. and 40 $\mu\mu$g. per ml.; the lowest normal level was 100 $\mu\mu$g. per ml. The patients of Badenoch (1954), Chalmers and Boheimer (1954), and Chanarin, Elmes, and Mollin (1958) had normal serum levels, except one low normal level (120 $\mu\mu$g. per ml.) described by Badenoch (1954). Tests of vitamin-$B_{12}$ absorption, using the methods of serum levels, liver uptake, and faecal excretion, were all found to give results within normal limits by Badenoch (1954), Chalmers and Boheimer (1954), Kidd and Mollin (1957), and Chanarin, Elmes, and Mollin (1958). Hawkins and Meynell (1958) found vitamin $B_{12}$ ineffective in the treatment of the macrocytosis, but obtained a response in five patients with the megaloblastosis due to anticonvulsant drugs; three of these patients also needed

folic acid. One patient of Kidd and Mollin (1957) had a reticulocyte response of 4·8 per cent. to only one dose of 100 $\mu$g. of vitamin $B_{12}$. A 14 per cent. reticulocyte response was recorded by Hawkins and Meynell (1954), but there was no response in the haemoglobin level. One of Badenoch's (1954) cases responded, and one of Rhind and Varadi's (1954) patients showed a slight response. Webster's (1954) patient who was taking phenytoin showed a slight but definite response in the reticulocyte count, and the haemoglobin level rose from 24 per cent. to 80 per cent. after a small blood transfusion (1¼ pint) and cyanocobalamin therapy. Ryan and Forshaw (1955) recorded one patient with an 11·8 per cent. reticulocytosis and an increase of nearly 50 per cent. in haemoglobin, but they had withdrawn phenytoin. Chanarin, Elmes, and Mollin (1958) found no effect on the utilization of vitamin $B_{12}$ by *Euglena* in various concentrations of primidone. A perusal of these published studies, and of the review of the subject by Stokes and Fortune (1958), has not revealed any serial measurements of vitamin-$B_{12}$ absorption.

### *Technique*

Tests of radioactive vitamin-$B_{12}$ absorption were performed by the method of Schilling, Chatanoff, and Korst (1955). Each test involved the oral administration of a dose of the radioactive vitamin ($^{58}$Co-labelled vitamin $B_{12}$, approximately 1·0 mc) after a night of fasting and without breakfast. Urine collection was begun when the dose was given. Three hours later 1,000 $\mu$g. of ordinary vitamin $B_{12}$, was given by intramuscular injection. (This acts as a 'flushing' or saturating dose, so that radioactive material which has been absorbed is excreted in the urine.) A complete 24-hour specimen of urine was collected, and samples measured for radioactivity. The total excreted radioactivity was then compared with measurements of a standard solution of the administered material, in order to calculate the percentage of the dose which was excreted. In the following descriptions the percentage excretion of vitamin $B_{12}$ is referred to as 'absorption' unless otherwise stated; it is only a convenient way of stating the results, and is the terminology used by other writers. The technique briefly described above was used by Berlyne, Liversedge, and Emery (1957). Each batch of radioactive vitamin $B_{12}$ was tested in known cases of pernicious anaemia, to show that absorption was less than 2 per cent. of the dose in such cases. The tests on the eight patients were done with many different batches of material, indicating that the results did not depend on any particular batch. Any drugs that the patients were taking were also given at the usual times on test days. When stating the dosage of drugs, use is made of abbreviations such as phenytoin 60 mg. $\times$ 3 (60 mg. taken three times daily).

### *Case Histories and Results of Investigations*

*Case 1.* A single woman aged 36 was admitted to hospital on 11.2.58 because of increasing pallor, recent anorexia, and a tendency to diarrhoea. She had had epilepsy since childhood. For many years she had taken phenobarbitone, and

for the last 18 months phenytoin 90 mg. ×3 and primidone 250 mg. ×4. Examination revealed mucosal pallor and hypertrophy of the gums. There were no other abnormal signs. The initial haematological findings were: haemoglobin, 6·3 g. per 100 ml.; red cells, 1,500,000 per cu. mm.; colour index, 1·42; packed cell volume, 17 per cent.; mean corpuscular haemoglobin concentration, 31 per cent.; mean corpuscular volume, 121 cu. $\mu$; mean corpuscular

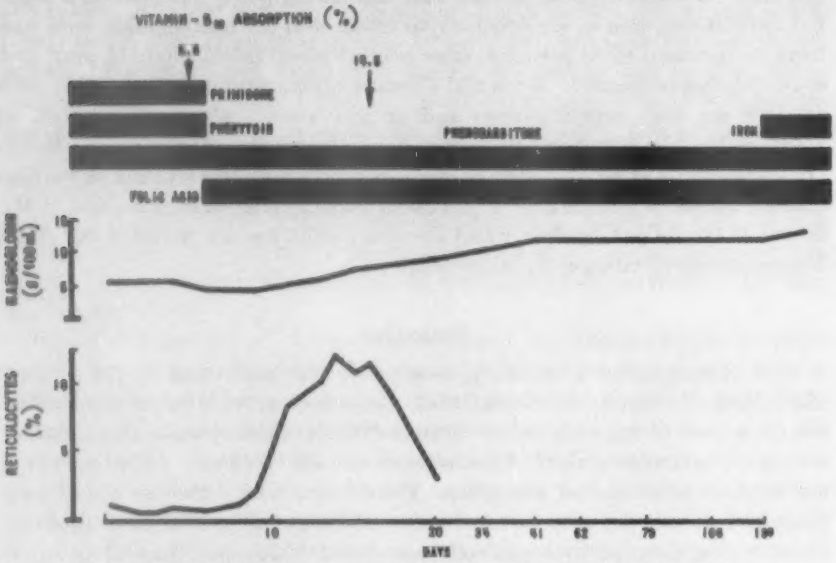


FIG. 1. The results of treatment and the Schilling tests in Case 1.

diameter, 7·9 $\mu$; white cells, 2,500 per cu. mm. (polymorphs 38·5 per cent., lymphocytes 58·3 per cent., monocytes 0·5 per cent. eosinophils 2 per cent.); platelets, 109,000 per cu. mm.; reticulocytes, 1·6 per cent. Blood films showed marked anisocytosis and poikilocytosis, with occasional punctate basophilia. Marrow examination showed a megaloblastic red-cell series, with many proerythroblasts and abnormal metamyelocytes.

Other investigations gave the following results: direct Coombs test, negative; faecal urobilinogen excretion, 0·43 mg. in 24 hours; serum bilirubin, 0·3 mg. per 100 ml.; serum albumin, 3·7 g. per 100 ml.; serum globulin, 1·8 g. per 100 ml.; thymol turbidity, 0·8 units; serum alkaline phosphatase, 6·0 King–Armstrong units; blood urea, 36 mg. per 100 ml. Absorption tests and other metabolic studies showed: serum carotene, 15 $\mu$g. per 100 ml.; serum vitamin A, 10 $\mu$g. per ml.; vitamin-A absorption (serum levels at 30-minute intervals): 20, 50, 62, 44, and 24 $\mu$g. per ml.; glucose tolerance (blood levels at 30-minute intervals after 50 g. of glucose by mouth): 105, 130, 155, 140, 120, and 110 mg. per 100 ml.; faecal fat, 19 per cent. of dried weight of faeces. A fractional test meal showed normal amounts of free gastric acid. A barium meal and follow-through showed only a slight dilatation of several segments of the ileum.

*Treatment and absorption tests* (Fig. 1). Six days after the patient's admission a vitamin-$B_{12}$ absorption test was performed, necessitating the administration of 1,000 $\mu$g. of cyanocobalamin. The next day phenytoin and primidone were stopped, and phenobarbitone, 60 mg. ×3, given alone as an anticonvulsant. On

the same day 10 mg. of folic acid were given by intramuscular injection, and the same dose was repeated on the two days following. Twenty mg. of folic acid were given orally each day throughout the rest of the period of study. A reticulocyte response of nearly 25 per cent. followed folic-acid treatment. The colour index fell to 1·0, and there was a steady increase in haemoglobin. The patient's condition continued to improve. She was seen on six occasions in the next four months. The haemoglobin increased to 12·1 g. per 100 ml.; the mean corpuscular haemoglobin concentration fell to 30, 27, and 28 per cent., and the colour index to 0·9 and 0·87 per cent. She was then given ferrous gluconate in addition to the continuous therapy with phenobarbitone and oral folic acid. The haemoglobin levels were over 12·5 g. per 100 ml. when she was seen three months later, and in 1959 the cure has been maintained.

The vitamin-$B_{12}$ absorption tests showed an absorption of 5·8 per cent. on 16.2.58, and 11 days later, on 27.2.58, 14·5 per cent.

*Case 2.* A man of 34 years had suffered from epilepsy since childhood. His symptoms were dyspnoea on excretion, anorexia, colicky abdominal pains, and occasional vomiting during the previous three months. He was taking phenobarbitone, 60 mg.×3, and phenytoin, 45 mg.×3. The phenytoin had been taken very regularly for about 18 years. For some years he had noticed increasing pallor. A few small ulcers had developed on the buccal mucosa and tongue just before his admission to hospital on 24.5.58. At this time he had diarrhoea, which proved to be due to a *Shigella Sonnei* infection and was rapidly cured by sulphaguanidine. Examination revealed a severe degree of pallor, and hypertrophy of the gums. He also had an arrested hydrocephalus and hemianopia. There were no signs of subacute combined degeneration of the cord, or of liver disease. The initial haematological findings were: haemoglobin, 5·6 g. per 100 ml.; red cells, 1,650,000 per cu. mm.; colour index, 1.15; packed cell volume, 16 per cent.; mean corpuscular haemoglobin concentration, 35 per cent.; white cells, 3,300 per cu. mm. (polymorphs 57·5 per cent., lymphocytes 29 per cent., monocytes 12 per cent., eosinophils 1 per cent.); one nucleated red cell per 200 white cells; platelets abundant. Marrow examination showed megaloblastic maturation, with marked hyperplasia of red-cell precursors at all stages. There were numerous giant metamyelocytes.

Other investigations gave the following results: first fat-balance study (8.6.58 to 13.6.58): 3·4 g. fat daily excreted on an intake of 75 g. fat; second fat-balance study (2.7.58 to 7.7.58): 2·6 g. daily excreted on an intake of 50 g. fat. A fractional test meal produced abundant free gastric acid. A barium meal and follow-through gave normal results. Neurological and general radiological investigations had been made previously, and were not repeated.

*Treatment and absorption tests* (Fig. 2). Phenytoin, 45 mg.×3, was continued throughout until 7.7.58, when phenobarbitone, 30 mg.×3, was continued alone and maintained indefinitely. The first Schilling test was on 2.6.58, and after this 100 $\mu$g. of cyanocobalamin were given daily except on the days of the tests, when 1,000 $\mu$g. were given. Folic acid, 15 mg. daily by intramuscular injection for three days, and at the same time 10 mg.×3 by mouth, was started on 15.6.58. This oral dose was subsequently given every day except for the five days 14.7.58 to 19.7.58. One hundred mg. of an intrinsic factor preparation were given orally with the third Schilling test on 21.6.58.

Schilling tests were performed on the following days: (1) 2.6.58, while the patient was receiving phenytoin and phenobarbitone; (2) 13.6.58, again with phenytoin and phenobarbitone, and during vitamin-$B_{12}$ therapy; (3) 15.6.58, with phenytoin, phenobarbitone, folic acid, vitamin $B_{12}$, and intrinsic factor; (4) 21.6.58, with phenytoin, phenobarbitone, folic acid, and vitamin $B_{12}$;

(5) 8.7.58, with phenobarbitone, folic acid, and vitamin $B_{12}$; (6) 17.7.58, with phenobarbitone and vitamin $B_{12}$. The results of these tests showed an excretion of 2·6 per cent., 2·8 per cent., 7 per cent., 7 per cent., 7·8 per cent., and 9·1 per cent., in that order.

The first response to treatment with vitamin $B_{12}$ was a rapid but moderate reticulocytosis, with a slight increase in haemoglobin. Folic acid then produced a more obvious rise in haemoglobin, and a maximum reticulocytosis of 22 per cent. The patient declared that after therapy with vitamin $B_{12}$ was begun he

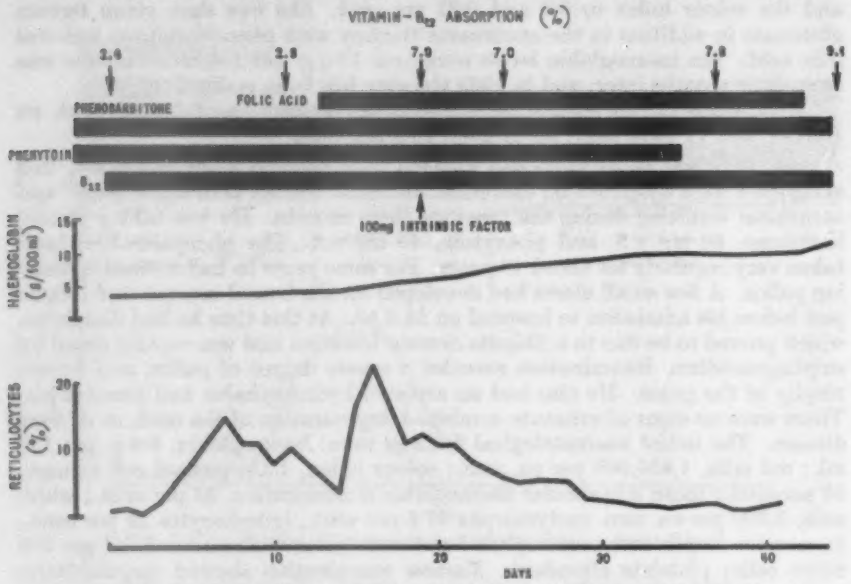


FIG. 2. The results of treatment and the Schilling tests in Case 2.

felt much better. Later, when folic acid was given, he said that it had been 20 years since he felt so well. For over 10 years he had become used to pallor, and soon he developed a ruddy complexion. The anorexia, dyspnoea, and other symptoms were relieved completely. On 30.9.58 the haemoglobin was 12·7 g. per 100 ml., and on 28.10.58 14·5 g. per 100 ml.; the colour index was 0·91. Improvement was maintained throughout 1959; phenytoin was given without ill-effects, and in December 1959 the haemoglobin was 14·8 g. per. 100 ml. The patient was still taking folic acid, 10 mg. × 3.

*Case 3.* A woman aged 46 years was admitted to hospital on 11.10.58. She had been epileptic since the age of 11 years. Until 15 years ago she had been given phenobarbitone, and for the next 14 years phenytoin. During the year before admission she had been taking primidone, 250 mg. × 5. She complained of palpitation on exertion and swelling of the ankles. Pallor was the only abnormal sign. The initial haematological findings were: haemoglobin, 9·5 g. per 100 ml.; colour index, 1.29; red cells, 2,359,000 per cu. mm.; packed cell volume, 32 per cent.; mean corpuscular haemoglobin concentration, 30 per cent.; mean corpuscular volume, 127 cu. $\mu$; mean corpuscular diameter, 8·0 $\mu$; white cells, 6,900 per cu. mm. (polymorphs 55 per cent., lymphocytes 39 per cent., monocytes 6 per cent.). Blood films showed marked anisocytosis and poikilocytosis, with some polychromasia. Examination of the bone marrow showed

megaloblastic changes in the red-cell series, but these changes were not conspicuous.

A fractional test meal with histamine produced no free gastric acid, and a quinine-resin ('diagnex') test produced an excretion of fluorescent material equivalent to a total excretion of less than 5 $\mu$g. of quinine. Although there was achlorhydria, the Schilling tests indicated that the patient could absorb vitamin $B_{12}$. A barium meal and follow-through gave normal results. A five-day fat-balance test showed 91 per cent. absorption. After a dose of 350,000 units of

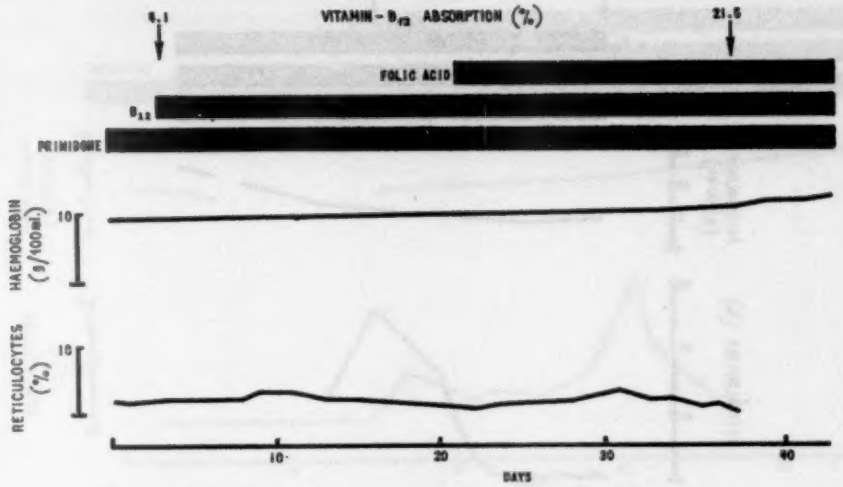


FIG. 3. The results of treatment and the Schilling tests in Case 3.

vitamin A the serum levels at 30-minute intervals were 25, 24, 60, 66, and 48 $\mu$g. per ml. Radiology of the chest and skull, examination of the cerebrospinal fluid, and air encephalography gave normal results. The erythrocyte sedimentation rate was 13 mm. in one hour (Wintrobe).

*Treatment and absorption tests* (Fig. 3). The previous treatment with primidone, 250 mg. $\times$ 5, was continued throughout the study and up to the time of writing. No other anticonvulsant was given. On 27.10.58 the patient had 1,000 $\mu$g. of cyanocobalamin with the first Schilling test, and after the test 100 $\mu$g. was given daily. On 14.11.58 folic acid, 15 mg., was given intramuscularly each day for eight days, and after this 10 mg. each day by mouth. Because she was not severely anaemic, only a slight reticulocyte response was measured. Haemoglobin levels gradually rose, and there was much improvement in well-being. When she was last seen on 16.9.59 the haemoglobin was 12·4 g. per 100 ml., and white cells 5,800 per cu. mm. (polymorphs 53 per cent., lymphocytes 42 per cent., monocytes 4·2 per cent., eosinophils 0·5 per cent.). She still took primidone and folic acid.

The first Schilling test resulted in an excretion of 4·1 per cent. of the dose. The patient was taking primidone only. The second test, 34 days later, when she was having primidone, folic acid, and vitamin $B_{12}$, resulted in 21·5 per cent. excretion.

*Case 4.* A woman aged 34 had been epileptic for 20 years. For eight years she had been treated with phenytoin and phenobarbitone. These drugs were

then discontinued, and she was given primidone, 250 mg. × 4. For three or four months before the present study she complained of anorexia, constipation, loss of weight, and dyspnoea on exertion. On 15.8.58 examination showed pallor, slight smoothness of the tongue, and sluggish ankle reflexes. There were no other abnormal signs. The initial haematological findings were: haemoglobin, 6·6 g. per 100 ml.; colour index, 1·16; packed cell volume, 21 per cent.; mean corpuscular haemoglobin concentration, 33 per cent.; white cells, 7,800 per cu. mm.;

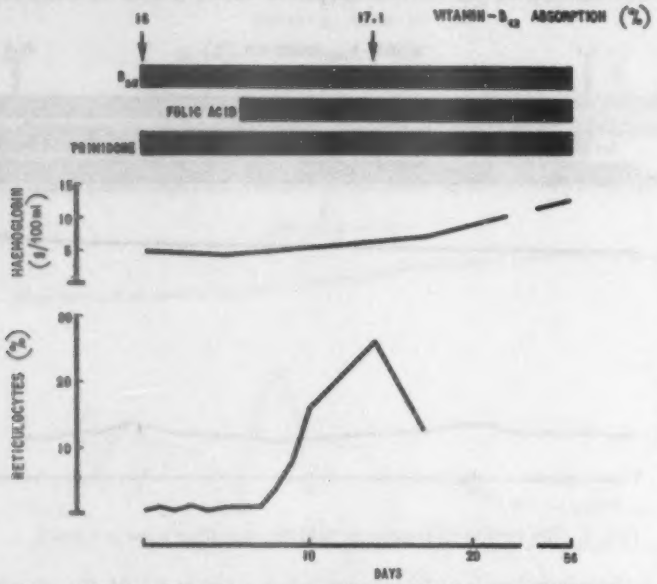


FIG. 4. The results of treatment and the Schilling tests in Case 4.

nucleated red cells, 2 per 200 white cells; platelets, 90,000 per cu. mm. The blood film showed marked anisocytosis and poikilocytosis. Marrow examination showed a megaloblastic red-cell series, with some normoblasts. There were many giant metamyelocytes and promyelocytes.

A fractional test meal showed free acid. No fatty-acid crystals were detected in the faeces. The serum calcium was 9·3 mg. per 100 ml., serum phosphate 3·7 mg. per 100 ml., serum vitamin A 21 μg. per ml., and serum carotene 20 μg. per 100 ml. Serum albumin was 3·7 g. per 100 ml., globulin 2·5 g. per 100 ml., bilirubin 0·2 mg. per 100 ml., thymol turbidity 1·2 units, serum alkaline phosphatase 7·5 King–Armstrong units, and blood urea 32 mg. per 100 ml.

*Treatment and absorption tests* (Fig. 4). Primidone was continued throughout the tests. On 27.8.58 the first Schilling test was performed, and after this the patient was given cyanocobalamin, 100 μg. intramuscularly, each day. Folic acid, 15 mg. intramuscularly, was given daily from 2.9.58, and 10 mg. × 3 orally from 6.9.58. She has been taking folic acid and primidone since that time. There was no reticulocyte response to cyanocobalamin; folic acid produced a good response. By 10.11.58 the haemoglobin had increased to 12·6 g. per 100 ml., and the colour index was 0·88. The first Schilling test, on 27.8.58, showed 16 per cent. excretion, and the second, on 9.9.58, 17·1 per cent. excretion.

*Case 5.* A woman aged 33 was admitted to the ward in December 1958. She had suffered from epilepsy for 13 years. In 1945 she had iron-deficiency anaemia. When seen in June 1957 she had been taking primidone, 250 mg. × 4, for six months. The haemoglobin, tested because of previous anaemia, was 12.4 g. per 100 ml. In July 1958 there were new signs and symptoms. She complained of anorexia and occasional vomiting, was pale, and had cheilosis and facial pigmentation resembling chloasma. The menstrual periods were normal. The

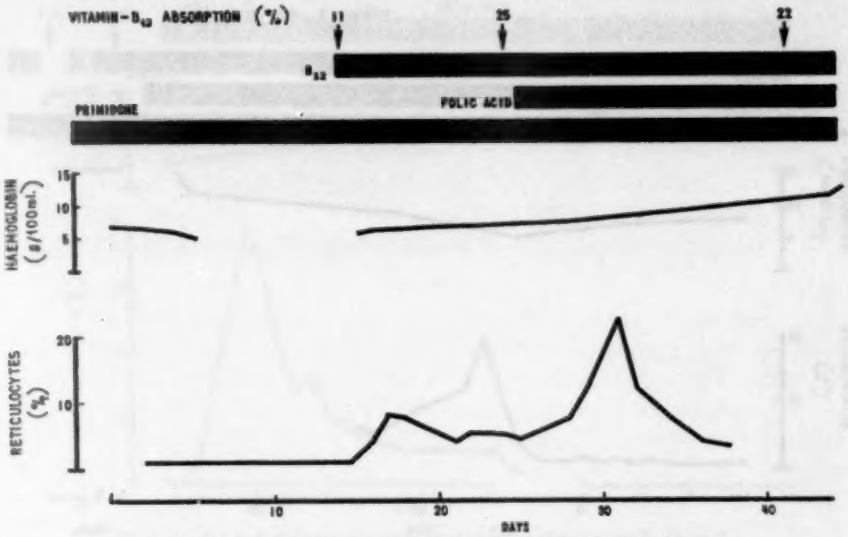


FIG. 5. The results of treatment and the Schilling tests in Case 5.

haemoglobin was 10.1 g. per 100 ml., and colour index 0·88, and she was treated with a parenteral vitamin-B mixture and intramuscular iron. During the next few months the pigmentation faded, but the anaemia did not respond. In November 1958 the haemoglobin was 8 g. per 100 ml., and colour index 1·2.

The patient was admitted for further investigation on 13.12.58. Examination revealed no new signs. The initial haematological findings were: haemoglobin, 6·8 g. per 100 ml.; colour index, 1·39; white cells, 5,100 per cu. mm. (polymorphs 79 per cent., lymphocytes 19 per cent., monocytes 2 per cent.). Blood films showed macrocytosis, anisocytosis, and poikilocytosis. Bone-marrow examination showed a grossly megaloblastic red-cell series and abnormal metamyelocytes.

A fractional test meal produced large quantities of free gastric acid; a fat-balance study with a daily intake of 100 g. fat showed an excretion of 3·8 g. per day over three days. Liver-function tests showed serum albumin 4·5 g. per 100 ml., globulin 1·9 g. per 100 ml., serum bilirubin 0·4 mg. per 100 ml., thymol turbidity 1·0 unit, and serum alkaline phosphatase 7·5 King–Armstrong units. A barium meal and follow-through gave normal results.

*Treatment and absorption tests* (Fig. 5). Primidone, 250 mg. × 4, was given uninterruptedly throughout the tests and since. On 29.12.58 a Schilling test was performed, followed by 100 $\mu$g. of cyanocobalamin daily. A reticulocyte response of 8 per cent. was obtained. Folic acid was begun on 9.1.59 in a dosage of 10 mg. × 3 by mouth. A reticulocyte response of 23 per cent. resulted. There was a gradual rise of the haemoglobin level to 13·3 g. per 100 ml. on 1.5.59.

Vitamin-$B_{12}$ absorption tests resulted in 11 per cent. excretion on 29.12.58, while the patient was receiving primidone; 25 per cent. on 8.1.59, with primidone and cyanocobalamin; and 22 per cent. on 25.1.59 with folic acid, primidone, and cyanocobalamin.

*Case 6.* A man, aged 32 in 1958, had suffered from infantile hemiparesis; after the age of 15 he had had grand mal epilepsy, and had been treated with phenobarbitone for some years, and with phenytoin, 90 mg. × 3, since 1948. He

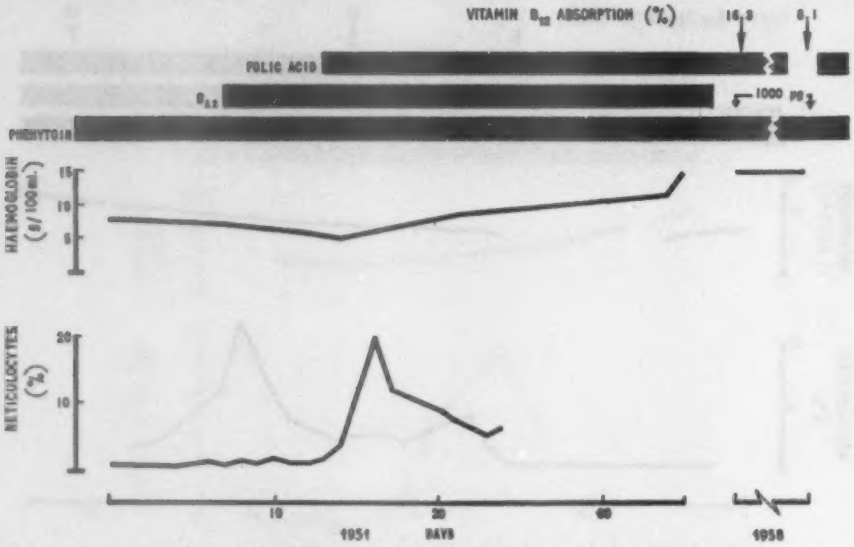


FIG. 6. Case 6. The results of treatment in 1951, and the two Schilling tests performed in 1958. The break in the record between the two tests indicates the lapse of three months.

attended hospital in 1951, when severe anaemia was noticed. Apart from a mild hemiparesis there were no other abnormal signs. Haematological investigations in 1951 revealed haemoglobin 7·4 g. per 100 ml. Blood films showed anisocytosis, poikilocytosis, and macrocytosis. Marrow examination showed a megaloblastic picture, with numerous intermediate forms. Large metamyelocytes were present, and some double- or triple-nucleated megaloblasts. A few normoblasts were seen. Two fractional test meals with histamine gave plenty of free gastric acid on both occasions. The fat balance was normal. Radiographs of the spine and pelvis were normal. The serum calcium was 9.8 mg. per 100 ml., serum phosphate 4·4 mg. per 100 ml., and serum alkaline phosphatase 5·5 King–Armstrong units.

In 1951 his condition was not recognized as anaemia caused by phenytoin, but he was given cyanocobalamin, 100 µg. daily. This produced a negligible reticulocytosis, and the haemoglobin level continued to fall to a level of 4·4 g. per 100 ml. Folic acid was then given, and produced a reticulocyte response of 21 per cent. From then onwards the haemoglobin rose to normal levels. Since 1951 the patient had been having phenytoin, and folic acid 20 mg. daily. When seen in November 1958 he was quite well except for occasional fits. The haemoglobin levels had been regularly maintained at 14·8 g. per 100 ml. since 1951.

*Treatment and absorption tests* (Fig. 6). A Schilling test was performed on 19.11.58, while the patient continued his usual doses of phenytoin and folic acid.

He excreted 16.3 per cent. of the dose of vitamin $B_{12}$. Three months later, on 13.2.59, the folic acid was discontinued, and five days later, while he was taking phenytoin only, a second Schilling test showed 8·1 per cent. excretion. He then continued his usual therapy. Fig. 6 shows the treatment and its results in 1951. Near the end of the chart can be seen the two Schilling tests. Their results

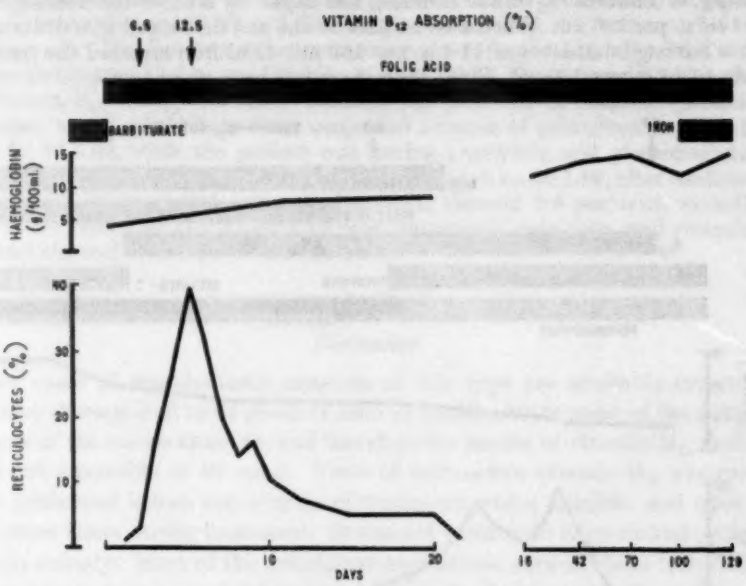


FIG. 7. The results of treatment and the Schilling tests in Case 7.

are reversed compared with the other cases. At the time of the first test the patient was receiving folic acid. At the second test he had been without folic acid for five days.

*Case 7.* A woman of 31 years was admitted to hospital on 31.10.58. For more than two years she had suffered from mental depression. Large quantities of barbiturates had been taken regularly during this period. For one month before admission there had been increasing lethargy and dyspnoea on exertion. There was no history of diarrhoea or dietary deficiency. Examination showed severe anaemia. The initial haematological findings were: haemoglobin, 4·1 g. per 100 ml.; red cells, 950,000 per cu. mm.; colour index, 1·55; packed cell volume, 13 per cent.; mean corpuscular haemoglobin concentration, 30 per cent., white cells, 3,400 per cu. mm. (polymorphs 82 per. cent., lymphocytes 16 per cent., monocytes 2 per cent.); platelets, 54,000 per cu. mm.; reticulocytes, 1·4 per cent. The bone marrow showed extreme megaloblastic hyperplasia, with many proerythroblasts and frequent giant metamyelocytes.

A 'diagnex' test showed the presence of plenty of free gastric acid; serum albumin was 4·1 g. per 100 ml.; serum globulin 1·9 g. per 100 ml., serum bilirubin 0·6 mg. per 100 ml., thymol turbidity 1·5 units, serum alkaline phosphatase 6·5 King–Armstrong units, and serum prothrombin 100 per cent. A barium gastrointestinal series was normal, and there was nothing to suggest steatorrhoea. A two-day faecal output contained 15 g. of fat. The serum vitamin A was 11 mg. per 100 ml., serum carotene 30 $\mu$g. per 100 ml., serum

calcium 9·0 mg. per 100 ml., and serum inorganic phosphate 4·7 mg. per 100 ml. Other investigations showed nothing relevant.

*Treatment and response* (Fig. 7). Barbiturates were discontinued immediately. On 31.10.58 the patient was given 20 mg. of folic acid intravenously, followed by 20 mg. orally each day. This was the only treatment except the two doses of 1,000 $\mu$g. of vitamin $B_{12}$ on the Schilling test days. By 20.2.59 the haemoglobin was 14·6 g. per 100 ml. When seen on 22.4.59 she had developed iron deficiency, with a haemoglobin level of 11·4 g. per 100 ml. Oral iron restored the haemoglobin to 14 g. per 100 ml. Since then there has been no anaemia.

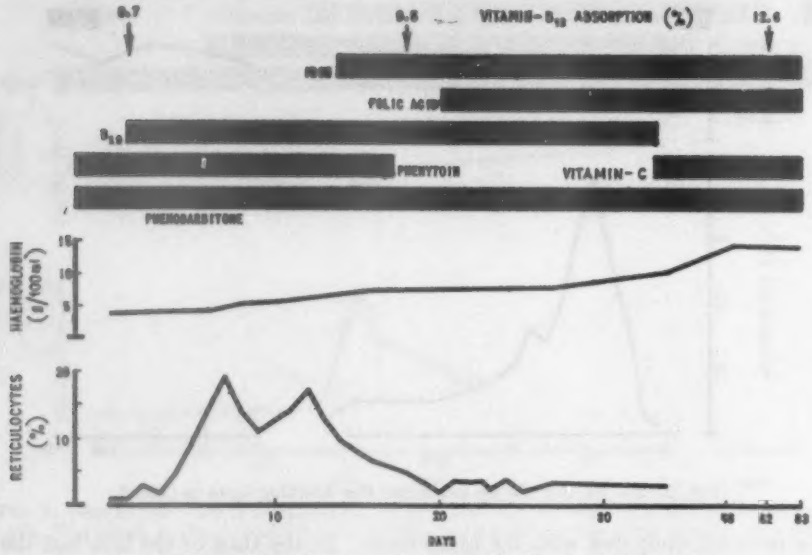


FIG. 8. The results of treatment and the Schilling tests in Case 8.

*Case 8.* A man of 52 years had had a 'stroke' in 1949, followed by epileptic attacks. A second 'stroke' in 1951 had produced dysphasia. The epilepsy had been treated with phenobarbitone, 45 mg. × 3, and phenytoin, 90 mg. × 3, since 1949. He was admitted to hospital on 8.1.59 complaining of dyspnoea, pallor, anorexia, loss of weight, and constipation. Examination revealed dysphasia, hemiparesis, and pallor. The initial haematological findings were: haemoglobin, 3·7 g. per 100 ml.; colour index, 1·0; packed cell volume, 13 per cent.; mean corpuscular haemoglobin concentration, 32 per cent.; mean corpuscular volume, 99 cu. $\mu$; mean corpuscular diameter, 7.5 $\mu$; white cells, 2,650 per cu. mm. (polymorphs 78 per cent., lymphocytes 18 per cent., monocytes 4 per cent.); platelets, 21,000 per cu. mm.; reticulocytes less than 1 per cent. Marrow examination showed a megaloblastic red-cell series, with giant metamyelocytes.

A fractional test meal with histamine showed achlorhydria. Serum iron was 300 $\mu$g. per 100 ml. Blood urea was 30 mg. per 100 ml., serum albumin 4·1 g. per 100 ml., serum globulin 2·1. g. per 100 ml., serum bilirubin 0·7 mg. per 100 ml., thymol turbidity 1·2 units, and serum alkaline phosphatase 5 King–Armstrong units. The direct Coombs test gave a negative result.

*Treatment and absorption tests* (Fig. 8). Phenytoin and phenobarbitone were given in the same dosage as before. Vitamin $B_{12}$ was started on 9.1.59 with a

Schilling test, and was continued at a dosage of 100 $\mu g$. daily. This produced a reticulocyte response of 19 per cent. On 28.1.59 oral folic acid, 20 mg. daily, was begun, but only a slight rise in the reticulocyte count was observed. Ferrous gluconate was also given to this patient, beginning on 21.1.59. Phenytoin was discontinued on 25.1.59, but the phenobarbitone was given throughout. When discharged from hospital on 4.2.59 the patient was given folic acid, 20 mg. per day, phenobarbitone, 90 mg. $\times$ 3, ferrous gluconate, 0·6 g. $\times$ 3, and ascorbic acid, 100 mg. $\times$ 3. The last two substances were given empirically. By 25.2.59 the haemoglobin was 13·2 g. per 100 ml., and on 1.4.59 12·4 g. per 100 ml.

Vitamin-$B_{12}$ absorption tests excluded the presence of classical pernicious anaemia, which might have been suspected because of achlorhydria. The first test, on 10.1.59, while the patient was having phenytoin and phenobarbitone, showed an excretion of 8·7 per cent. The second test, on 26.1.59, after continuous vitamin-$B_{12}$ therapy but without phenytoin, showed 9·5 per cent. excretion. On 11.3.59, while he was taking folic acid, phenobarbitone, iron, and vitamin C, the test showed a 12·6 per cent. excretion.

### *Discussion*

Few cases of megaloblastic anaemia of this type are available for study. Curative therapy had to be given as soon as practicable to most of the patients because of the severe anaemia, and therefore the results of vitamin-$B_{12}$ therapy were not assessable in all cases. Tests of radioactive vitamin-$B_{12}$ absorption were performed before any change of treatment where possible, and again at opportune times during treatment. It was not possible to have control patients in such a study. Most of the descriptive and clinical details about this kind of anaemia have been recorded by other writers. In the cases described above all the data have been included so that they can be used for future reference. The responses to vitamin $B_{12}$ and to folic acid, and the results of the first and last Schilling tests in each patient, are shown in Table I, with the interval of time between the tests. Drug therapy, and the period of time during which each drug had been taken before admission to hospital, are also recorded. It can be seen that in seven cases the absorption of radioactive vitamin changed. This change was relatively slight in Case 8. In six cases the change was considerable, and varied from a twofold to a fivefold increase in absorption. This appears to be a significant increase and unlikely to be a chance finding.

A possible objection to the view that absorption has been increased is that a patient 'saturated' with vitamin $B_{12}$ might excrete a larger proportion of the dose than a patient who is 'unsaturated', and whose tissues might fix more vitamin. This is almost certainly true, but we are dealing with very large doses of vitamin $B_{12}$. In Case 2, for example, a continuous rise in radioactive vitamin excretion occurred with every test, though tissue saturation would be expected to have taken place quite early with the constant large doses of cyanocobalamin. It can be assumed that in these circumstances the rise in excretion implies an increased absorption. In Case 6 it is impossible to hold the view that tissue unsaturation would materially affect the result, because the test during folic-acid therapy (16·3 per cent. absorption) was made three months

before the test without folic acid (8·1 per cent. absorption), and the only doses of vitamin $B_{12}$ were those given at the test times. Any tendency of the patient to excrete more vitamin when 'saturated' would have been reflected in a rise, not a fall, of the level of excretion at the second test. It can further be suggested that variations in response to the Schilling test in 'normal' persons might have affected the results. In the writer's experience such gross changes as were seen in the present series of anaemic patients are unusual in normal subjects. In order to test this question further, Schilling tests were performed on seven patients

TABLE I

| *Case number* | *Sex* | *Age (years)* | *Drugs* | *Duration of administration of drugs* | *Response to folic acid* | *Response to vitamin $B_{12}$* | *First Schilling test (excretion %)* | *Time interval* | *Final Schilling test (excretion %)* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 36 | Phenobarbitone<br>Phenytoin<br>Primidone | Many years<br>18 months | ++ | Not assessable | 5·8 | 11 days | 14·5 |
| 2 | M | 34 | Phenobarbitone<br>Phenytoin | Irregularly<br>18 years | ++ | + | 2·6 | 46 days | 9·0 |
| 3 | F | 46 | Phenobarbitone<br>Phenytoin<br>Primidone | 15 years<br>1 year<br>1 year | + | Nil | 4·1 | 34 days | 21·5 |
| 4 | F | 34 | Phenytoin<br>Phenobarbitone<br>Primidone only | 8 years<br>8 months | ++ | Nil | 16·0 | 13 days | 17·1 |
| 5 | F | 33 | Primidone | 2 years | ++ | + | 11·0 | 26 days | 22 |
| 6 | M | 32 | Phenytoin | 10 years | ++ | Nil | 16·3 on folic acid | 90 days | 8·1 without folic acid |
| 7 | F | 31 | Barbiturates | 2 years | ++ | Not assessable | 6·5 | 5 days | 12·5 |
| 8 | M | 62 | Phenytoin<br>Phenobarbitone | 10 years | + | ++ | 3·7 | 90 days | 12·6 |

who were taking no drugs and had no evidence of anaemia or gastrointestinal disease. The tests were made before, and then a number of days after, the administration of folic acid 10 mg. three times daily, including the same dosage on the day of the second test. The results are shown in Table II. In this control series of patients it can be seen that excretion, or absorption, decreased in four, and in three there was a slight increase, though very little compared with results in the anaemic patients. The only appreciable change was a decrease. The possibility that residual amounts of radioactive material were being excreted by the anaemic patients during the second and subsequent tests was excluded by testing urine to ensure 'background' levels before re-testing. In most cases repeated dosage of cyanocobalamin should have 'flushed out' residual radioactive vitamin. In these circumstances it was found that about three or four days after a test there was usually no detectable radioactive excretion in the urine. Similar findings have been obtained in other patients, after tests both with oral and with intravenous radioactive vitamin $B_{12}$. It was thought most improbable that a build-up of radioactive excretion could have affected results in the series of anaemic patients. The possible influence of anti-epileptic drugs on fixation of vitamin $B_{12}$ in the tissues is a factor to be considered, but the present investigation does not illuminate this problem. If such a fixation took place, the first doses of vitamin $B_{12}$ would be excreted to a lesser degree than subsequent doses.

Assuming that an increased absorption of vitamin $B_{12}$ in fact occurred in seven patients with megaloblastic anaemia caused by anticonvulsant drugs, it

remains to discuss the possible mechanisms for this change. All seven cases responded to folic acid. Cases 2, 5, and 8 showed a response to cyanocobalamin, and in Cases 1 and 7 this response could not be assessed. In Case 6 it appears that the only variable factor at the time of the tests, apart from vitamin $B_{12}$, was folic acid. The result implies that vitamin $B_{12}$ taken with folic acid and phenytoin was absorbed more easily than when no folic acid was taken, and this in spite of the fact that the patient must have been fully saturated with folic acid after taking it regularly for seven years. The simple explanation, that folic acid might facilitate absorption of vitamin $B_{12}$, does not necessarily follow

TABLE II

| *Subject* | *Excretion (%) 1st test* | *Interval on folic acid (days)* | *Excretion (%) 2nd test* |
|---|---|---|---|
| 1 | 24 | 26 | 20 |
| 2 | 6 | 10 | 7·5 |
| 3 | 14 | 10 | 8 |
| 4 | 4 | 10 | 3 |
| 5 | 6 | 7 | 7·3 |
| 6 | 3 | 10 | 2 |
| 7 | 4·5 | 4 | 5·5 |

in other cases. In Case 1 the factor increasing absorption might have been the withdrawal of primidone and phenytoin or the administration of folic acid. In Case 2 the maximum absorption could not have been due to the presence of folic acid in the lumen of the gut, because at the time of the last test the patient was not receiving folic acid. The last two tests might have shown increased absorption because phenytoin had been stopped; but absorption on the third and fourth tests had already risen from 2·6 per cent. to 7 per cent. in spite of phenytoin. Vitamin $B_{12}$ and folic acid were being given throughout. No definite change was observed with intrinsic factor. In Case 3 the rise in absorption occurred during primidone therapy, and might have been effected by vitamin $B_{12}$ or by folic acid. In Case 5 also primidone was given without a break, yet absorption rose from 11 per cent. to 22 per cent. Folic acid or vitamin $B_{12}$ could have been responsible, but absorption increased before folic acid as well as being maintained after it. In Case 7 the increase in absorption from 6·5 per cent. to 12·5 per cent. might have been caused by folic acid, vitamin $B_{12}$, or the withdrawal of barbiturates. In Case 8 the results, if significant, could have been occasioned by folic acid, vitamin $B_{12}$, or the withdrawal of phenytoin.

The main observations were that absorption rose with or without withdrawal of the offending anti-epileptic drugs; that folic acid could have been responsible for the change in all cases, except that in Case 5 absorption increased with vitamin $B_{12}$ alone; and that, because of the nature of the tests, vitamin $B_{12}$ itself might have been responsible for change except in Case 6, in which the direction of the change was reversed. The conclusion to be drawn is that either folic acid or vitamin $B_{12}$ itself could be responsible for changes in the body which result in a higher level of vitamin-$B_{12}$ absorption.

The writer has not been able to find any record of an interrelationship of vitamin-$B_{12}$ and folic-acid absorption and, in particular, has seen no work on

this subject in relation to the anaemias caused by anticonvulsant drugs. Girdwood (1959), in a recent comprehensive review of the role of folic acid in blood disorders, did not mention this point. He drew attention to the fact that both vitamin $B_{12}$ and folic acid are concerned in the transfer of one-carbon units, and that in this regard vitamin $B_{12}$ is concerned with methyl group syntheses, folic acid with hydroxymethyl and other group syntheses. Folic-acid co-factor is concerned with the formation of purines and pyrimidines, while vitamin $B_{12}$ is concerned with the formation of nucleosides. The failure to produce deoxyribosenuclease may lead to megaloblast production. Whatever the biochemical explanation, he made it clear that folic acid and vitamin $B_{12}$ act as 'partners' in the process of normal erythropoiesis. An interesting point is that in the megaloblastic anaemia of pregnancy the level of serum vitamin $B_{12}$ is often low. This anaemia is not due to malabsorption, though dietary deficiency may play a contributory part even in Britain (Thompson, 1957). It would be interesting to test vitamin-$B_{12}$ absorption in such cases, before and after curative folic-acid therapy, in the light of the present results obtained in anaemia due to anticonvulsant drugs. Similar studies would be interesting in dietary deficiencies and malabsorption syndromes. We probably know little about the dependence of absorption of different substances on the 'conditioning' effects of other deficiencies.

The effect of vitamin-$B_{12}$ therapy in cases of megaloblastic anaemia due to anticonvulsant drugs is variable. It frequently produces a partial haematological response, and occasionally a cure. Three of the patients here reported showed a reticulocytosis with cyanocobalamin. Other authors have quoted similar cases. A dietary deficiency has been suggested to explain the 'deficiency' in such patients, especially in those with low serum levels of vitamin $B_{12}$. The fact that vitamin $B_{12}$ alone may produce a haematological response has been held to indicate that there must be available folic acid in the body, because both are necessary for haemopoiesis. If folic acid is being 'blocked' in the tissues by anticonvulsant drugs, one must assume that massive doses of vitamin $B_{12}$ can overcome this block to some extent. Folic acid must also be capable of overcoming any biochemical 'block' which exists, because its administration is completely successful without the withdrawal of the anticonvulsant.

Apart from any possible dietary deficiency of vitamin $B_{12}$ in patients with anaemia due to anticonvulsant drugs, it is possible that in some cases, if not in all, a relative defect in absorption may be present. This might be sufficient to lower serum levels to low-normal or subnormal, as described by other investigators, and to produce a tissue deficiency of the vitamin. The results of the present tests appear to support this hypothesis, because, although all the levels of absorption were in the normal range, they nearly all rose during the clinical improvement that accompanied therapy. It may be that low-normal tissue levels and suboptimal levels of absorption are insufficient when the tissues contain anticonvulsants which are blocking folic acid, just as they are probably insufficient for some pregnant women who have folic-acid and vitamin-$B_{12}$ deficiency with normal absorption.

It appears possible that this relative change in vitamin-$B_{12}$ absorption might be caused by an effect of anticonvulsant drugs on the intestinal mucosa. Such an effect, if not direct, could be metabolic, and might even be mediated through a change in folic-acid metabolism. It is an interesting fact that phenytoin commonly produces a hypertrophy of the gums. A possible suggestion is that phenytoin and other anticonvulsants may also have functional effects on the intestinal wall. Phenytoin, phenobarbitone, and primidone have a general structural similarity, and in this respect they resemble folic acid. Girdwood and Lenman (1956) suggested this affinity might result in a competitive interference with certain metabolic processes mediated by folic acid. Since the function of folic acid is so closely related to that of vitamin $B_{12}$, it appears possible that such interference in the gut wall might affect the absorption of vitamin $B_{12}$. Other features frequently met with in patients with this type of anaemia point to gastrointestinal disturbance, namely anorexia, diarrhoea, and border-line levels of fat absorption or definite steatorrhoea. The symptoms described in the present paper also included abdominal pains, vomiting, constipation, and loss of weight. One patient showed areas of slight dilatation of the ileum. The levels of vitamin-$B_{12}$ absorption were, of course, not those which would be expected in frank steatorrhoea.

In patients with anaemia due to anticonvulsant drugs gastrointestinal investigations usually prove negative. The results of crude tests such as glucose tolerance, fat balance, and barium studies, though relevant to the hypothesis of functional changes in the gut wall, prove nothing by being normal. Badenoch (1954) described findings of histologically normal material from gastric biopsy. No studies of small-intestinal mucosa in such patients appear to have been made. Studies of the effects of anticonvulsants on the absorption of vitamin $B_{12}$ in normal persons and, if changes were found, of the influence of folic acid, would be a useful extension of the investigation. Serial absorption studies in patients with similar anaemias might also be revealing.

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## *Summary*

Eight patients who had megaloblastic anaemia due to anticonvulsant drugs were studied. The effects of treatment with vitamin $B_{12}$ and folic acid are recorded. Tests of absorption of radioactive vitamin $B_{12}$, using the Schilling technique, were performed before curative therapy and at various times during it. In seven cases it was found that a considerable increase in vitamin-$B_{12}$ absorption took place. This change was related to folic-acid therapy in six

cases. It is suggested that a functional change occurs in the gut wall in some patients taking anticonvulsant drugs, and that this change results in a relative defect in vitamin-$B_{12}$ absorption, which may be sufficient to produce a deficiency in such patients.

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# NEEDLE BIOPSY OF THE PLEURA IN THE DIAGNOSIS OF PLEURAL EFFUSION[1]

*A Report of 118 Cases*

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With Plates 26 and 27

Pleural biopsy carried out specifically for diagnostic purposes has been the subject of several publications in recent years. Sutcliff, Hughes, and Rice (1954) described 21 cases in which pleural specimens had been obtained by so-called open pleural biopsy involving rib resection, and Breckler, Hoffman, Hill, Hensler, and Hukill (1956) reported 16 cases in which the pleural sample was removed by surgical biopsy without rib resection. The first report of needle biopsy of the pleura was that of DeFrancis, Klosk, and Albano (1955), who described six cases; a further 20 cases in which the specimen had been obtained in this way were added by Heller, Kellow, and Chomet (1956). The two methods were compared by Donohoe, Katz, and Matthews (1958), who reported their findings in 78 needle and 54 surgical biopsies. They advocated a limited thoracotomy in patients in whom needle biopsy failed to give a positive result. A large series of 152 cases in which pleural effusion was the main clinical problem was published in this country by Mestitz, Purves, and Pollard (1958), and another 78 cases were briefly reported by Karlish (1959 *a*). A small series of 20 cases was added by Leggat (1959), and 26 more were recently described by Pagel and Goldfarb (1960).

The total number of cases published to date probably exceeds 500, but the justification for describing a further series is threefold. As Pagel and Goldfarb (1960) remarked, there is as yet no unanimity of opinion about the usefulness of the method or its precise role in diagnosis, and the publication of this comparatively large series may go some way towards defining it. In the second place, the present series is concerned solely with patients in whom pleural effusion was the presenting and predominant clinical problem. Finally, it probably represents a better sample of the general hospital population than do some of the earlier series (Mestitz, Purves, and Pollard, 1958), which were heavily weighted in favour of younger male patients.

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### *Patients Studied and Methods*

Our study covers 118 patients, with a total of 136 biopsies obtained during the years 1957–60. Most of the patients came from the medical wards of the Reading group of hospitals, which serves a population of 300,000. Of the 118 patients 63 were male, aged 21 to 84 years (mean 55), and 55 female, aged 14 to 85 years (mean 48). The 'Harefield' needle described by Abrams (1958) has been used in all these cases in preference to the Vim Silverman needle, which is widely employed in America; its main advantage is that the biopsy specimen can be obtained simultaneously with the aspiration of fluid, thus avoiding a further thoracocentesis. A satisfactory specimen consists of a cube or strip of tissue about 2 to 3 mm. long. On naked-eye examination it consists of red intercostal muscle with white or grey pleural tissue at one end. In health the pleural layer will be thin and glistening; if diseased it will usually be thickened, greyish-white, or grey and dull. It is often possible to tell at the time of operation whether or not a satisfactory specimen has been obtained. In the present series four specimens were found to be inadequate. Complications were few and minor in character: a small pneumothorax was caused in three patients, but was due to faulty technique, as became clear from later experience. Immediately after removal the specimen is wrapped in thin paper to prevent loss of small fragments of tissue, and is placed in fixative, which has been 10 per cent. formol saline in all cases. After fixation, dehydration, and clearing, paraffin impregnation is carried out overnight, and the specimens are then embedded in small flat-bottomed glass tubes measuring $\frac{3}{4} \times \frac{1}{2}$ inch, which are broken after the wax has cooled. Serial sections are cut and mounted in sets of five, a number of sections being discarded between each mounted series. The usual biopsy specimen, by this technique, has yielded about 10 to 15 slides, each having five sections.

### *Results*

A considerable range of histological findings has been encountered. In some cases a layer of normal pleura was present. Abnormal findings were reported as: thickened pleura without active inflammatory change; acute or chronic inflammatory reaction not suggestive of any specific aetiology; changes regarded as suggestive or diagnostic of tuberculosis; and finally features regarded as suggestive or diagnostic of malignant disease. The positive diagnoses in the present series have been limited (apart from the recognition of non-specific inflammatory change) to tuberculosis and malignant disease. Pagel and Goldfarb (1960) in their series reported one case in which the findings were believed to be suggestive of a rheumatic aetiology in a man with a 16-year history of rheumatoid arthritis. The present series includes three patients known to be suffering from rheumatoid arthritis, but in none of them were any histological features present which would have enabled a diagnosis to be made from the pleural biopsy.

In the tuberculous cases the histological findings have often, but not always, been well-defined. Plate 26, Fig. 1 shows a typical lesion. Caseation is often present, and usually there is a well-marked epithelioid-cell reaction. Lymphocytes have generally been rather scanty. Langhans-type giant cells are usually to be found, but Pagel and Goldfarb (1960) have warned against a too ready acceptance of their presence as evidence of tuberculosis: in one of their patients with rheumatoid arthritis they found typical Langhans giant cells in the pleural biopsy specimen, but there was no other evidence of tuberculosis, and they were satisfied that this was not, in fact, the diagnosis. In general, the criteria for a diagnosis of tuberculosis are the same in pleural biopsy specimens as elsewhere, and, while the diagnosis in the present series was usually clear, there were three cases in which the findings, though suggestive, were not regarded as sufficiently clear-cut to warrant a definite diagnosis. In these three cases, however, the final diagnosis was of tuberculosis. Plate 26, Fig. 2 shows a tuberculous focus in which the appearances are not so clear-cut as they are in the earlier illustration, but were nevertheless believed to justify a positive diagnosis.

In the malignant cases the histological appearances showed wide variation. So far as the positive cases are concerned the two extremes were, on the one hand, the presence of a few isolated cells which were recognized as malignant more by the application of the criteria used in exfoliative cytology than by histological criteria—and, on the other hand, the presence of well differentiated tumour deposits from which it was possible to give an opinion about the probable site of the primary tumour. Plate 27, Fig. 3 shows a collection of cells with large hyperchromatic nuclei, which were identified as malignant. The patient was later found to have a squamous carcinoma of the bronchus, the diagnosis being confirmed by bronchial biopsy. Plate 27, Fig. 4 shows an unusually good example of a well differentiated malignant deposit, and it was possible to suggest in this case that the tumour was a secondary deposit from a mammary carcinoma, as in fact it was. Figs. 5 and 6 (Plate 27) show examples of secondary deposits in the pleura from carcinoma of a bronchus: in Fig. 5 from a squamous tumour, and in Fig. 6 from an oat-cell tumour. The appearances are very similar to those encountered in positive bronchial biopsies. Difficulties in interpretation have been encountered, as Mestitz, Purves, and Pollard (1958) mentioned, in cases in which the pleural cells are actively proliferating in the presence of an inflammatory process. In these circumstances the pleural cells may show a good deal of variation in shape and size; bizarre forms are not uncommon, and the cells may be found in mitotic division. A useful distinguishing feature in such cases is that these pleural cells are not found to be ramifying among the intercostal muscle fibres, while this is often the case with malignant deposits.

Apart from the biopsies which have been recognized as suggestive or diagnostic either of tuberculosis or malignant disease, the other abnormal specimens have been regarded as indicative of acute or chronic inflammatory change without specific features. In some pyogenic chest conditions typical acute

inflammatory changes have been seen, and in other cases the picture has been that of a more chronic inflammation, with fibrosis and new blood-vessel formation. In one case of empyema a prominent feature in the biopsy was the presence of large numbers of pigment-laden macrophages.

In Table I the results of 136 pleural biopsies performed in 118 patients are compared with the final diagnosis. The results of these biopsies have shown

TABLE I

*Results in the Present Series*

| *Final diagnosis* | *Pleural biopsy findings* | | | | |
|---|---|---|---|---|---|
| | *Positive* | *Probable* | *Non-specific* | *Inadequate* | *Total* |
| Tuberculosis | 20 | 4 | 5 | 3 | 32 |
| Malignancy | 22 | 3 | 14 | 1 | 40 |
| Empyema | .. | .. | 13 | .. | 13 |
| Rheumatoid arthritis | .. | .. | 3 | .. | 3 |
| Pneumonia | .. | .. | 12 | .. | 12 |
| Heart failure | .. | .. | 4 | .. | 4 |
| Pulmonary infarct | .. | .. | 7 | .. | 7 |
| Others | .. | .. | 7 | .. | 7 |
| | 42 | 7 | 65 | 4 | 118 |

that positive diagnoses of tuberculosis and malignant disease have been made in a high proportion of cases; if the positive and probable results are taken together, 24 out of 32 cases of tuberculosis (75 per cent.) were correctly diagnosed, and 25 out of 40 cases of malignant disease (62·5 per cent.).

## *Discussion*

The earlier reports of the use of this technique have stressed the value of the method in the diagnosis of tuberculous pleural effusion. The finding of positive evidence of tuberculosis in such cases has always been a difficult matter, and several recent surveys have shown that when bacteriological

TABLE II

*Bacteriological Diagnosis of Tuberculous Pleural Effusion*

| *Authors* | *Total cases* | *Positive cases* |
|---|---|---|
| Emerson (1954) | 40 | 18 (45·0%) |
| Roper and Waring (1955) | 141 | 51 (36·2%) |
| Mestitz and Pollard (1958) | 84 | 24 (28·6%) |
| Karlish (1959 *b*) | 83 | 28 (33·7%) |

methods alone are employed the proportion of cases yielding positive results cannot be expected to exceed 45 per cent. (Table II). In the survey of 83 pleural effusions notified as tuberculous in Reading in the years 1953–7 and reported by Karlish (1959 *b*), 28 (33·7 per cent.) were found to be positive after thorough bacteriological investigation. In practically all these cases culture and guinea-pig inoculation of pleural fluid was undertaken, as well as culture

and guinea-pig inoculation of gastric washings where sputum was not obtainable. In contrast with these results, in the present study 24 out of 32 cases finally diagnosed as tuberculous (75 per cent.) were classified as definitely or probably positive on pleural biopsy. A combination of all available methods, including pleural biopsy, gave evidence of tuberculosis in 30 of the 32 cases, or 94 per cent. The age distribution in this tuberculous group is of some interest; there were 16 male patients, aged 17 to 78 years (mean 43), and 16 female patients, aged 14 to 47 years (mean 29). The high mean age of men seen in recent years with tuberculosus pleural effusion further emphasizes the need for laboratory confirmation, as malignant disease must be considered in the differential diagnosis of many of these patients.

The proportion of cases finally diagnosed as malignant which have been correctly identified by pleural biopsy is lower than in the tuberculous group, both in our series and in those of other authors. Thus Donohoe, Katz, and Matthews (1958) had 40 per cent. of successes, and Mestitz, Purves, and Pollard (1958) reported 65 per cent. of diagnostic results. In the present series, if positive and probable results are grouped together, a correct diagnosis was established by pleural biopsy in 25 out of 40 cases, a rate of 62·5 per cent.

Further analysis of the malignant group shows, as would be expected, that bronchial carcinoma forms the largest number of cases; there were 27 such patients, 21 men and six women, making a higher proportion of women than usual. A positive pleural biopsy was obtained in 14 of the 27 patients (52 per cent.). Pagel and Goldfarb (1960) reported 13 positive results in 26 patients, of whom 20 were finally diagnosed as having malignant disease, an incidence of 65 per cent. correct diagnoses. They pointed out that of these 20 patients only nine had been previously diagnosed by the finding of malignant cells in sputum or pleural fluid, and that the majority had given negative results on bronchoscopy. In our series cytological examination for malignant cells in sputum or pleural fluid was done in 23 of the 27 patients, and was positive in 13 cases, a rate of 48 per cent., slightly lower than that obtained by pleural biopsy (52 per cent.). Bronchoscopy was performed in 20 patients, and was positive 12 times (60 per cent.). The remaining seven patients were either unfit or too old for bronchoscopy. All methods of examination, including pleural biopsy, cytology of sputum and pleural fluid, and bronchoscopy, gave a histological diagnosis in 23 of the 27 patients (85 per cent.).

The next most frequent condition in the malignant group was pleural effusion in women with a history of mammary carcinoma. A definite rather than a presumptive diagnosis is important here because of the more hopeful methods of treatment now available for some patients. In the present series there were seven such patients, and pleural biopsy showed malignant deposits in six.

This investigation has shown that confirmation of the diagnosis can be expected in a high proportion of tuberculous or malignant effusions. It is clear, however, that the method is to be regarded as an additional means of examination, and not as a substitute for any existing diagnostic procedure. Only the employment of all available methods will give the maximum number of

correct diagnoses. False positive results should be very rare. The danger of making a false positive diagnosis of malignancy by misinterpretation of the appearance of proliferating pleural cells has already been mentioned, but with experience this should be avoided. In the present series a suspicion of oat-cell carcinoma was wrongly aroused in the case of a man with pneumonia and pleural effusion; this has been the only misleading case up to the present time. As with all laboratory methods, there have been some disappointing failures; one of them concerned a girl of 17 years with a large pleural effusion and a strongly positive tuberculin test. Cultures of pleural fluid and of gastric washings were both positive, but two pleural biopsies were negative. A possible explanation of this may lie in the timing of the biopsy, for in the early stages only small-cell infiltrations may be present without the formation of characteristic tubercles, and in such cases the biopsy should be repeated a fortnight later. Another possible cause of failure to identify tuberculosis is previous antibacterial treatment, which may alter histological appearances, and it is desirable, therefore, that biopsy should be carried out in the course of the first diagnostic aspiration of fluid.

There were other unexpected results. A man aged 60 developed bilateral staphylococcal pneumonia after resection of a gangrenous ileum; the organisms were resistant to most antibiotics, and he did not respond to treatment. As small quantities of purulent fluid were aspirated at both bases, pleural biopsy was carried out, and showed bilateral tuberculous changes. He received antituberculous drugs, and made an excellent recovery. A woman aged 64 complained of cough and pleuritic pain of eight days' duration. She had a large, slightly blood-stained pleural effusion, and a strongly positive Mantoux test. Routine pleural biopsy showed a well differentiated adenocarcinoma suggestive of mammary origin, and on further clinical examination slight nipple retraction and small palpable axillary glands were discovered. The presence of mammary carcinoma was later confirmed. Another notable case was that of a man aged 48, who had a persistent eosinophilic pleural effusion of several months' duration. One of the authors (A. J. K.) took a pleural biopsy specimen while demonstrating the method at a conference. The histological finding (Dr. A. Beemer) was oat-cell carcinoma.

As a result of the present study we believe that the value of pleural biopsy examination in cases of pleural effusion has been confirmed. It is suggested that it should form part of the programme of investigation in any effusion of doubtful aetiology, and that it will confirm the diagnosis in a high proportion of tuberculous and malignant cases.

We wish to thank Drs. P. Mestitz and A. C. Pollard for their help during the early stages of this work, and Drs. S. Gerhard and T. Lee, residents at Peppard Chest Hospital, for taking some of the biopsy specimens. We are grateful to our colleagues, both physicians and surgeons, who referred their patients to us.

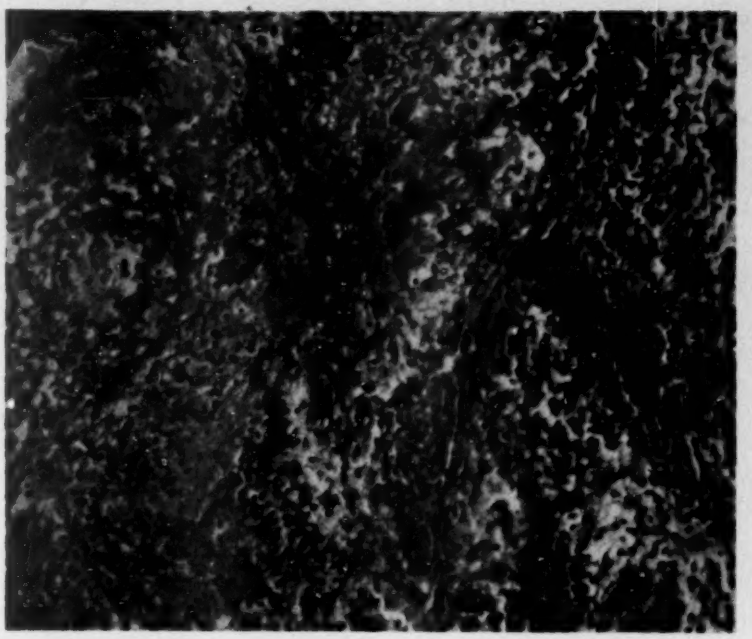

FIG. 1. Lesion typical of tuberculous pleurisy (×120)

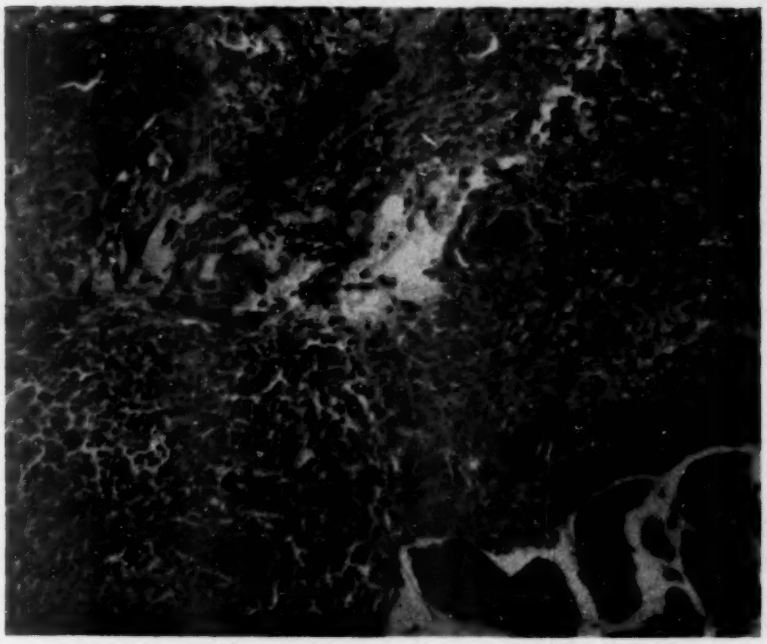

FIG. 2. Lesion suggesting tuberculous pleurisy (×120)

FIG. 3. Cells with hyperchromatic nuclei diagnostic of malignant disease (×500)

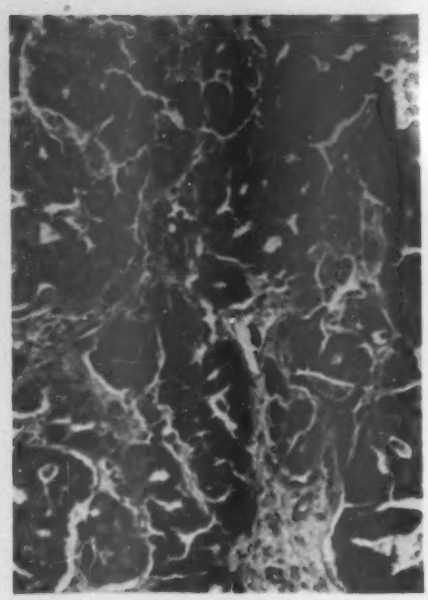

FIG. 4. Pleural metastasis from mammary carcinoma (×100)

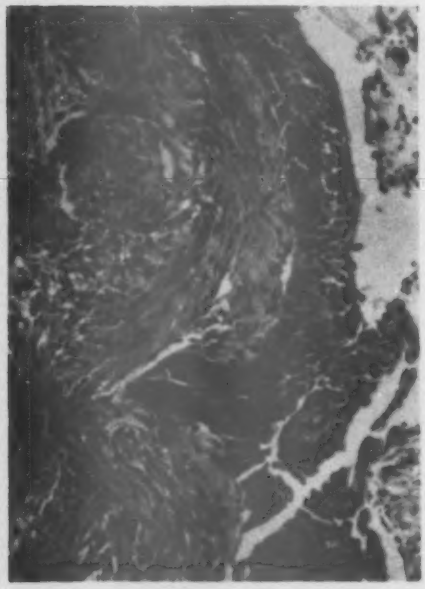

FIG. 5. Pleural metastasis from squamous bronchial carcinoma (×100)

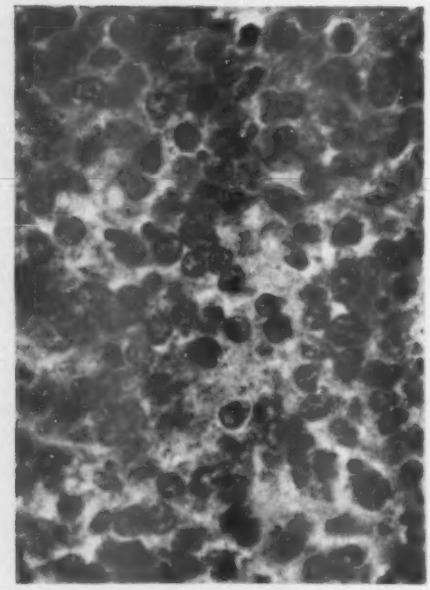

FIG. 6. Pleural metastasis from oat-cell bronchial carcinoma (×500)

*Summary*

The results of 136 pleural biopsies carried out in 118 patients are presented. Of 32 cases finally diagnosed as tuberculous, 21 were reported as positive and three as probable, a total incidence of 75 per cent. correct diagnoses. All methods of investigation, including pleural biopsy, gave definite evidence of tuberculosis in 30 of the 32 cases (94 per cent.).

Of 40 cases finally diagnosed as malignant, 22 were reported as positive and three as probable, a total rate of 62·5 per cent. correct diagnoses. In the malignant group there were 27 cases of bronchial carcinoma with 14 positive biopsies, and seven cases of mammary carcinoma with six positive biopsies. A combination of methods, including cytology of sputum and pleural fluid, bronchoscopy, and pleural biopsy, gave a histological diagnosis in 23 of the 27 patients suffering from bronchial carcinoma (85 per cent.).

The investigations confirmed the impression of previous workers that the method is a valuable diagnostic aid in the diagnosis of pleural effusion. It is suggested that an effusion should not be regarded as of unknown origin unless pleural biopsy has been carried out, preferably during the initial aspiration of the chest.

Positive specific diagnoses have been limited to the recognition of tuberculous and malignant disease. Non-specific inflammatory appearances of both acute and chronic types have been seen. In three cases of pleural effusion associated with rheumatoid arthritis no specific histological features were noted on pleural biopsy.

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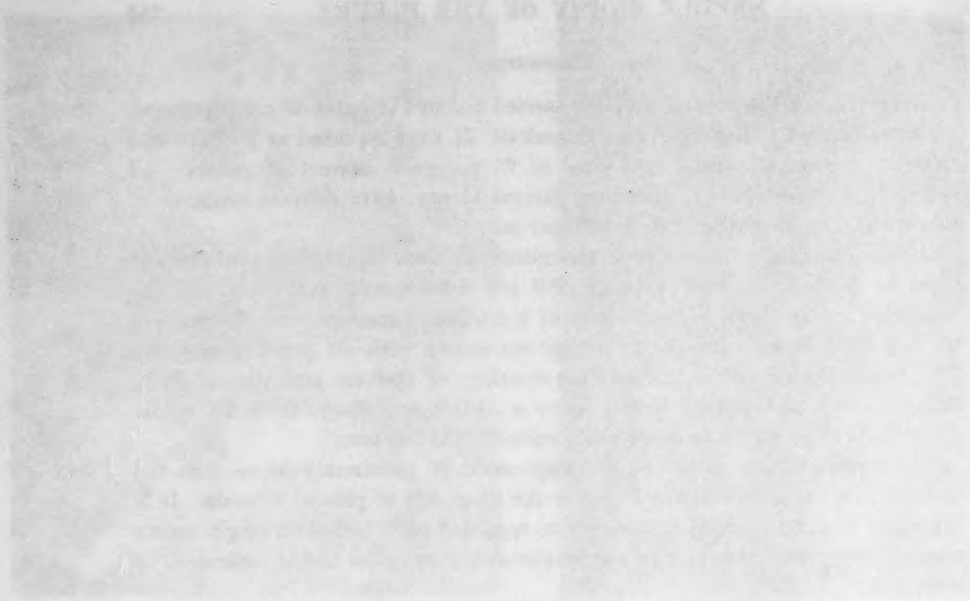

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# CHRONIC THYROTOXIC MYOPATHY[1]

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With Plates 28 and 29

Sixty-five years ago Bathurst (1895) published a case report of a man of 20 years who had thyrotoxicosis and severe muscular weakness and wasting. Since then about 60 other cases of chronic thyrotoxic myopathy have been reported, and from these and from five cases that we have recently had the opportunity of studying it is possible to provide a more comprehensive description of the clinical manifestations of the condition than has been available hitherto. The precise mechanism responsible for its development, however, remains unknown, as does the reason why certain thyrotoxic patients are so affected while others escape.

*Incidence.* The condition is one of considerable rarity, but it is impossible to say how often it complicates thyrotoxicosis. Four of the five cases that we have studied occurred in women in the fifth decade, and the fifth was an older man who was also a diabetic. Of the previously recorded cases, however, 60 per cent. have occurred in male and 40 per cent. in female patients. Thyrotoxicosis being so much more common in female than in male subjects, it would appear that men are specially prone to chronic thyrotoxic myopathy. The age range in the recorded female patients is 18 to 64 years with a mean of 43·1, and in the male patients 20 to 69 with a mean of 46·6, which is probably within the normal range of incidence of thyrotoxicosis.

*Duration of symptoms.* In all the five patients whom we have observed the history of thyrotoxicosis was short. In four cases muscular weakness first showed itself after thyrotoxicosis had been present for some weeks or months, while in Case 5 it developed at the same time as the symptoms of hyperthyroidism. In Case 1 thyrotoxicosis had been present for five weeks, while the myopathic symptoms had been evident for only a fortnight. In Case 2 symptoms of thyrotoxicosis had been noticeable for a year, the muscular weakness developing during the five weeks immediately preceding admission to hospital. In Case 3 thyrotoxicosis had been present for eight months and muscle weakness for three months, while in Case 4 thyrotoxicosis had been present for nine months and had been treated with neomercazole. In view of the unsatisfactory control so obtained, the drug was withdrawn before partial thyroidectomy. A severe exacerbation of thyrotoxicosis resulted, and with it profound muscle

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weakness and wasting developed. The fifth patient (who also had undiagnosed diabetes) had probably been thyrotoxic for seven months, and his muscle weakness was of similar duration.

The same pattern of behaviour is evident in the cases previously described, in most of which the history of thyrotoxicosis was of a year or less, while the myopathic symptoms had been evident for the same or a lesser period of time. It would appear, therefore, that patients with long-standing thyrotoxicosis are unlikely to develop myopathy; but the probable explanation is that only mild degrees of thyrotoxicosis are likely to remain long untreated, and myopathy is usually associated with very severe thyrotoxicosis.

*Severity of thyrotoxicosis.* All the five patients whom we have observed were severely thyrotoxic. Patient No. 1 had lost two stone in weight in five weeks, and had a basal metabolic rate of +66 per cent.; patient 2 had a basal metabolic rate of +72 per cent.; in patient 3 it was +77 per cent., and patient 4 was so violently thyrotoxic that it was impossible to obtain a satisfactory estimation. Patient 5 had lost over three stone in weight, and had a basal metabolic rate of +71 per cent. The patients previously recorded appear to have had thyrotoxicosis of similar severity, the basal metabolic rate in most cases being grossly elevated. In a minority, however, the rate has not been very greatly increased. The patient reported by Collings and Lienhard (1957) had a basal metabolic rate of only +28 per cent., but the 24-hour radio-iodine uptake was 81 per cent.; two patients of the group described by Millikan and Haines (1953) had basal metabolic rates of +32 and +36 per cent., but here again the radio-iodine uptake was very high at 92·8 per cent. in 24 hours in one case and 72·6 per cent. in the other, while the levels of protein-bound iodine were 10·0 $\mu$g. and 14.7 $\mu$g. per 100 ml. respectively. Thyrotoxic myopathy therefore appears to be a complication of severe thyrotoxicosis, and is unlikely to develop in patients with milder degrees of increased thyroid activity.

*Symptoms and signs resulting from the myopathy.* The distribution of the muscular weakness and wasting shows a striking constancy. Patient 1 noticed marked weakness of her legs, and fell down when trying to get on a bus. She was unable to lift her legs off the bed when lying flat, and was unable to get up from lying or kneeling without the help of her hands. Her quadriceps and the muscles of her upper arms showed marked wasting, and there was gross weakness of the neck and trunk flexors, the elbow extensors, the hip flexors, and the knee extensors. Patient 2 noticed that her thighs ached and felt tired and heavy when she was climbing stairs, and she had to assist the ascent with her hands. She also noticed that her arms became unusually tired when she was carrying cartridge boxes at her place of work. She had great difficulty in boarding a bus, and in getting out of a low chair, and she was unable to get up from the lying or kneeling position without using her hands. The muscles of her thighs, shoulder girdle, and upper arms were markedly wasted, and there was severe weakness of neck and trunk flexion, flexion and extension of the elbows, flexion of the thighs and knees, and dorsiflexion of the feet and toes. Patient 3 had noticed that her knees felt weak, and that she had difficulty in getting up from bending or kneel-

ing unless she used her hands. There was wasting of both quadriceps and deltoids, but no other abnormalities could be detected. Patient 4 was unable to get up from lying or kneeling, and had great difficulty in ascending stairs. The thighs, the deltoid muscles, and the interossei of the upper limbs were wasted, and muscle power throughout the body was markedly diminished, but neck and trunk flexion and flexion of the thighs were especially weak.

The cases previously recorded have shown a similar symptomatology and pattern of muscle weakness and wasting. Difficulty in walking (Bathurst, 1895; Starling, Darke, Hunt, and Brain, 1938; Parsons and Twort, 1939; Millikan and Haines, 1953; Melville, 1959), in climbing stairs (Parsons and Twort, 1939; Thorn and Eder, 1946; Millikan and Haines, 1953; Hoffenburg and Eales, 1956), in getting up from lying and kneeling (Bathurst, 1895; MacKenzie, 1940; Millikan and Haines, 1953; Hoffenburg and Eales, 1956; Melville, 1959), in standing (Collings and Lienhard, 1957), in sitting up (Starling, Darke, Hunt, and Brain, 1938; Bartels and Pizer, 1944; Thorn and Eder, 1946), in getting out of a chair (Bathurst, 1895; Millikan and Haines, 1953; Kite, McLintock, and Graves, 1954; Hoffenburg and Eales, 1956; Melville, 1959), in getting out of bed (Thorn and Eder, 1946), in turning over in bed (Bartels and Pizer, 1944), in climbing a ladder (Sanderson and Adey, 1949), in raising the arms (Thorn and Eder, 1946; Millikan and Haines, 1953; Collings and Lienhard, 1957), in wielding a hammer (Bartels and Pizer, 1944) and in combing the hair (Thorn and Eder, 1946; Millikan and Haines, 1953) have been among the symptoms reported. In nearly all the recorded cases weakness and wasting of the shoulder and pelvic girdles, of the quadriceps and upper arms, and of the small muscles of the hands has been the main feature, and weakness of neck and trunk flexion has been conspicuous.

Four of the patients we have observed (Cases 1 to 4) showed no alteration in the reflexes and no sensory loss, and the same is true of all recorded cases. One of our patients (Case 5) showed muscle fibrillation, and this has been reported by other observers (Ayer, Means, and Lerman, 1934; Starling, Darke, Hunt, and Brain, 1938; Parsons and Twort, 1939; Morgan and Williams, 1940; Sanderson and Adey, 1949; Quinn and Worcester, 1951; Kite, McLintock, and Graves, 1954; Melville, 1959).

In Case 5 of our group the picture was complicated by the presence of diabetes. The patient was grossly thyrotoxic, with tachycardia, tremor, exophthalmos, and a basal metabolic rate of +71 per cent. His legs were very weak, and he had great difficulty in walking and often fell down. He was unable to get up from lying and kneeling. Neck, trunk, and hip flexion, flexion and extension of the knees and elbows, and dorsiflexion of the feet were very weak. There was gross wasting of the shoulder girdles and upper arms, the buttocks and thighs, and the interossei of the hands. He thus presented the characteristic picture of chronic thyrotoxic myopathy, but it was thought that a diabetic neuropathy was also present, as he had a good deal of pain and paraesthesiae in all four limbs, and there was some sensory disturbance and loss of tendon reflexes.

*Response to prostigmine and curare.* Four of our five patients (Cases 1, 2, 4, and 5) were given an intramuscular injection of prostigmine in a dose of 1·0 to 2·0 mg., but in none of them did it produce any increase in power in the weakened muscles. The same finding has been recorded in many of the previous case reports (Morgan and Williams, 1940; Thorn and Eder, 1946; Bartels and Pizer, 1944; Zierler, 1951; Quinn and Worcester, 1951; Millikan and Haines, 1953;

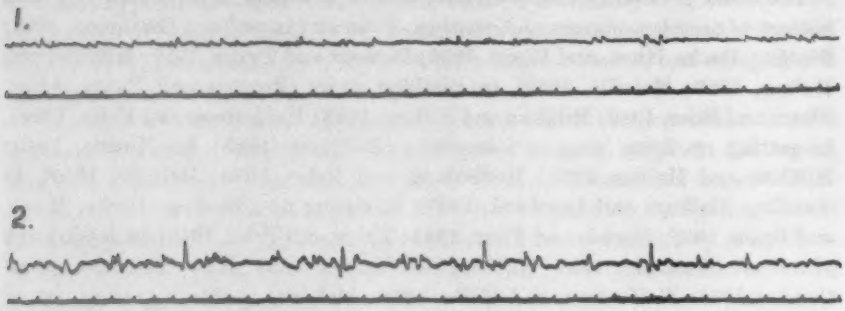


FIG. 1. Electromyograms from the rectus femoris in Case 1. (1) Before operation, showing greatly reduced motor-unit activity, with motor-unit potentials low in voltage and of disintegrated appearance. (2) Three and a half weeks after partial thyroidectomy, showing marked improvement but still some diminution of motor-unit action potentials.

Hoffenburg and Eales, 1956; Collings and Lienhard, 1957), but Sanderson and Adey (1949) and Melville (1959) considered that it produced slight temporary improvement in muscle power in the patients they observed, and Kite, McLintock, and Graves (1954) reported some reduction in fasciculation. Thyrotoxicosis is sometimes complicated by myasthenia gravis (Dudgeon and Urquhart, 1926; Thorn and Eder, 1946) and in such cases the muscle weakness is diminished by prostigmine (McEachern and Ross, 1942). In some cases the muscle weakness appears to be partly due to chronic thyrotoxic myopathy and partly due to myasthenia gravis (Prasad and Levi, 1955), and it seems likely that this was the explanation of the response to prostigmine in the case reported by Sanderson and Adey (1949). The curare test provides an additional means of differentiating between myopathy and myasthenia, myasthenic subjects being extremely sensitive to curare, which greatly increases their muscle weakness. We did not apply the curare test to any of our patients, but this was done by Millikan and Haines (1953) in eight of their cases; one-fifth of a curarizing dose of tubocurarine produced no increase of muscle weakness in any patient.

*Electromyography* from the right rectus femoris was carried out in Case 1, and showed greatly reduced motor-unit activity, the motor-unit potentials being low in voltage and of disintegrated appearance (Fig. 1). One month later (three and a half weeks after partial thyroidectomy) the record still showed diminution of motor-unit action potentials, but the abnormality was very much less marked. Electromyographic findings have been reported by other workers. Wegmann and Pipberger (1954) obtained a record similar to that shown in Fig. 1. After

treatment with methimazole there was only slight improvement, despite an increase in muscle mass. Sanderson and Adey (1949) found normal amplitude but shortened duration of spikes, and serial studies showed no change up to two months after partial thyroidectomy. Millikan and Haines (1953), however, reported normal tracings from three of their patients in whom electromyography was done, as did Melville (1959) in his patient.

*Muscle biopsy* was carried out in Cases 1, 2, 4, and 5. In Case 1 the rectus femoris showed moderate patchy atrophy of muscle fibres, with a few areas of fatty replacement (Plate 28, Fig. 2). The atrophied fibres showed an increased number of subsarcolemmal nuclei. In Case 2 a biopsy from the front of the thigh showed atrophy of muscle-fibres and sarcolemmal proliferation (Plate 28, Fig. 3). In Case 4 biopsy from the rectus femoris showed cellular aggregates consisting of lymphocytes and histiocytes between muscle-fibres (Plate 28, Fig. 4); the rectus abdominis in Case 5 showed atrophy (Plate 29, Fig. 5) and fatty replacement (Fig. 6) of muscle-fibres. Normal muscle biopsies have been reported by Morgan and Williams (1940) (from the neck), Millikan and Haines (1953), Kite, McLintock, and Graves (1954), Collings and Lienhard (1957), and Melville (1959), while other writers have described findings similar to those seen in our group (Morgan and Williams, 1940; Thorn and Eder, 1946; Quinn and Worcester, 1951; Adams, Denny-Brown, and Pearson, 1953; Hoffenburg and Eales, 1956). The histological changes seen in the muscles of patients suffering from chronic thyrotoxic myopathy are, however, non-specific, and similar appearances may be observed in other muscle disorders. They were in fact reported by Askanazy (1898) and Dudgeon and Urquhart (1926) in the muscles of thyrotoxic patients who had died, and who in life had not apparently shown any evidence of myopathy.

*The cerebrospinal fluid* was examined in only one of our five patients (Case 5), and no abnormality was present. Negative findings have been reported by other writers (Ayer, Means, and Lerman, 1934; Starling, Darke, Hunt, and Brain, 1938; Parsons and Twort, 1939; Hoffenburg and Eales, 1956).

*Potassium metabolism.* Periodic paralysis has been described in association with thyrotoxicosis (Dunlap and Kepler, 1931; Morrison and Levy, 1932; Brain, 1938), and in all six reported cases it resolved after partial thyroidectomy. There was nothing periodic about the muscular weakness in any of the five patients we studied; their serum-potassium levels were all within the normal range, while their electrocardiograms showed no evidence of potassium deficiency. The patient reported by Collings and Lienhard (1957) had been thyrotoxic for six months, and muscle weakness had been evident for three weeks. Though the serum-potassium level was normal, potassium abolished the muscle weakness, while glucose increased it. The muscle weakness disappeared with treatment of the thyrotoxicosis with radioactive iodine. We did not give potassium to any of the patients we studied.

*Creatine metabolism.* Creatine is synthesized in the liver from glycine and other amino-acids, and 95 per cent. of the total body creatine is normally present as phosphocreatine in muscle cells. It is required for muscular contraction, and

when so used is converted into creatinine and excreted in the urine. Normally no creatine is excreted in the urine, except in childhood and in women during menstruation. Creatinuria does however occur in starvation, in wasting diseases, in carbohydrate deprivation, in the febrile state, in diabetics, in thyrotoxicosis, and in muscular atrophies and dystrophies (Palmer, Carson, and Sloan, 1929). Thorn (1936) found creatinuria of the order of 210 mg. per 24 hours in 12 thyrotoxic patients at a basic metabolic rate of +50 per cent., but

*Maximum Urinary Creatinuria before Treatment of Thyrotoxicosis*

| | *Maximum level of creatinuria in 24 hours (mg.)* | *Creatininuria during same period (mg.)* |
|---|---|---|
| Case 1 | 330 | 420 |
| Case 2 | 142 | 694 |
| Case 4 | 177 | 1,103 |
| Case 5 | 175 | 1,520 |

in some of his cases of thyrotoxicosis it reached a level of 500 mg. per day. Shaffer (1908) reported creatinuria of up to 1·51 g. in 24 hours in a study of 43 cases of toxic goitre. The presence of creatine in the urine appears to be due to muscle wasting. Estimations of the urinary creatine and creatinine in chronic thyrotoxic myopathy are therefore not very meaningful as far as the muscle weakness is concerned, since such high levels are found in thyrotoxicosis without myopathy. The findings in the four cases we have studied are shown in the Table; it will be seen that they have in no instance exceeded the levels found in uncomplicated thyrotoxicosis. This accords with the findings of others who have investigated this aspect of chronic thyrotoxic myopathy (Morgan and Williams, 1940; Thorn and Eder, 1946; Sanderson and Adey, 1949; Zierler, 1951; Quinn and Worcester, 1951; Hoffenburg and Eales, 1956; Melville, 1959).

*Response to treatment.* Whether the thyrotoxicosis is treated surgically, with radioactive iodine, or with drugs of the thiouracil type, once normal thyroid function has been restored there is progressive improvement in the myopathy, and within a few months all muscle weakness and wasting disappears. Patients 1, 2, 3, and 4 were treated by partial thyroidectomy and afterwards with physiotherapy. In each of these four cases all subjective and objective evidence of myopathy had disappeared within three months of operation. Patient 3 developed both tetany and myxoedema after operation, but this did not seem to hinder recovery from the myopathy. Patient 4, in addition to physiotherapy, was given 25 mg. of 'durabolin' (nandrolone; nortestosterone phenylpropionate) by injection once each week, but this did nor appear to cause a more rapid recovery than that seen in Cases 1, 2, and 3. In previously reported cases the response of the myopathy to the control of the thyrotoxicosis has been equally satisfactory, however such control has been attained. But although the myopathic manifestations respond well, no myasthenic component or ophthalmoplegia has improved with restoration of normal thyroid function (Starling, Darke, Hunt, and Brain, 1938; Prasad and Levi, 1955).

Patient 5 was treated with methyl thiouracil, and complete control of his diabetes was obtained with tolbutamide. Despite the extremely satisfactory response as far as both the thyrotoxicosis and the diabetes were concerned, 15 months later there was still subjective and objective neurological abnormality, though very striking improvement had occurred. The slow improvement of the myopathy in this patient is attributed in part to its severity, but in the main to the fact that it was complicated by a diabetic neuropathy. Patients reported by MacKenzie (1940), Morgan and Williams (1940), Kite, McLintock, and Graves (1954), and Collings and Lienhard (1957) also had diabetes, but in all of these cases the neurological disorder appears to have been wholly myopathic, and the muscle weakness cleared up rapidly with control of the thyrotoxicosis.

*Similar myopathies.* There have been impressive reports of myopathies developing in association with steroid therapy, which appear very similar to those occurring in severe thyrotoxicosis, and clear up rapidly when steroid therapy is withdrawn, and sometimes when a change is made to a steroid not containing a fluorine radicle. Such myopathy has most commonly been seen in patients taking triamcinolone (Kendall and Hart, 1959; MacLean and Schurr, 1959; Williams, 1959), and has recently been reported as occurring in a patient treated with dexamethasone (Golding and Begg, 1960). Both these steroids contain the fluorine radicle, but Harman (1959) has described two such cases in patients taking prednisone. The mechanism involved in steroid myopathy, like that of thyrotoxic myopathy, is quite unknown, but it is more than possible that there is an aetiological link between the two conditions.

### *Case Reports*

*Case 1.* A married woman aged 42, employed as a part-time machine worker in a clothing factory, was admitted to the Queen Elizabeth Hospital, Birmingham, in October 1954. She had had no significant previous illness, and had one child aged seven years. For five weeks before admission she had been nervous, tearful, tremulous, and tired, and had had difficulty in sleeping, while a goitre had appeared, and she had lost 2 stone in weight. For two weeks her legs had felt weak, and when attempting to board a bus she had fallen down. On examination she was severely thyrotoxic. There was a moderate uniform goitre, slight asymmetrical exophthalmos, marked tremor of the outstretched hands, visible arterial pulsation in the neck, a regular tachycardia at 132 per minute, a blood-pressure of 130/60, and a hyperkinetic heart with an apical systolic murmur. The basal metabolic rate was +66 per cent. The blood count, liver function tests, and electrocardiogram were normal, as was also the chest radiograph apart from minor calcified tuberculous foci. Examination of the central nervous system showed marked symmetrical wasting of the quadriceps and upper arms. Disability was quite out of proportion to the extent of muscle wasting. When lying down the patient could not lift her legs off the bed. Trunk, neck, and hip flexion were very weak, and she was unable to get up from lying or kneeling without using her hands. Extension of the elbows and knees was also very weak. There was no fibrillation, no sensory loss, no alteration in the reflexes, and no cranial nerve involvement. An injection of prostigmine produced no improvement. The serum electrolytes, calcium, and phosphorus

were normal. An electromyogram from the right rectus femoris (Fig. 1) showed greatly reduced motor activity, with motor-unit potentials low in voltage and disintegrated in appearance. The tracing showed no evidence of myasthenia. The 24-hour urinary creatine and creatinine were 330 mg. and 420 mg. respectively on admisson. As a pre-operative measure the patient was given 200 mg. of methyl thiouracil and 2 ml. of Lugol's iodine daily from the time of admission, and within a week the creatinuria had disappeared. Partial thyroidectomy (Mr. D. M. Morrissey) was carried out 16 days after admission, and at the same time a muscle biopsy specimen was taken from the left quadriceps. When exposed, the degree of atrophy of this muscle was striking. Histologically the thyroid showed the usual appearances of thyrotoxicosis, while the quadriceps muscle biopsy showed moderated patchy atrophy of muscle fibres with a few areas of fatty replacement (Plate 28, Fig. 2). The atrophied fibres showed an increased number of subsarcolemmal nuclei. The patient made an uninterrupted recovery from the operation, and with physiotherapy all subjective and objective evidence of myopathy disappeared in three months. Electromyography one month after operation showed marked improvement, but the motor-unit action potentials were still diminished (Fig. 1).

*Case 2.* A married woman aged 47, a part-time worker in a cartridge factory, was admitted to the Queen Elizabeth Hospital, Birmingham, in February 1958. She had had no previous illness, and had two children aged 19 and 13. During the past year she had lost one and a half stone in weight, and had been troubled by trembling, palpitation, and lassitude. For five weeks she had been short of breath on exertion, and her ankles had tended to swell. Over a similar period of time she had noticed that her thighs ached, and felt tired and heavy when she was going upstairs, and she had to assist her ascent with her hands. She had difficulty in getting out of a low chair and in boarding a bus, and when carrying cartridge boxes at her place of work she noticed that her arms felt unusually tired. She was severely thyrotoxic, with marked tremor of the outstretched hands, a moderate uniform goitre, and exophthalmos. There was slight oedema of the ankles, but no other evidence of heart failure. The pulse was 120 per minute and regular, the blood-pressure 150/60, and the heart showed evidence of the hyperkinetic state but was otherwise normal. The basal metabolic rate was +72 per cent. The chest radiograph, blood count, liver function tests, and electrocardiogram were normal. On examination of the central nervous system there was wasting of the thighs, shoulder girdles, and upper arms. No fibrillation was evident, and there was no sensory loss, and no abnormality of the reflexes or cranial nerves. She was unable to get up from lying or kneeling without using her hands; neck flexion was almost absent, and flexion of the trunk and thighs was very weak, while there was also some weakness of flexion and extension of the elbows, flexion of the knees, and dorsiflexion of the feet and toes. An injection of 1·5 mg. of prostigmine had no effect on the muscle weakness. The serum electrolytes, calcium, and phosphorus were normal; the 24-hour urinary excretion of creatine was 142 mg., and of creatinine 694 mg. After two and a half weeks' rest, sedation, and Lugol's iodine, partial thyroidectomy (Mr. Victor Brookes) and biopsy from the right quadriceps were carried out. The patient was given physiotherapy for three months after operation, by which time all evidence of myopathy had disappeared and she had gained one and a half stone in weight, and was euthyroid and free from symptoms. Histologically the thyroid showed the usual appearances of toxicity, while the quadriceps muscle showed atrophy of muscle-fibres and proliferation of subsarcolemmal nuclei (Plate 28, Fig. 3).

*Case 3.* A married woman aged 36 was admitted to the Queen Elizabeth Hospital, Birmingham, in July 1959. She had had no significant previous illness, but for eight months had noticed excessive sweating, dyspnoea on exertion, intermittent diarrhoea, irritability, and loss of weight. Examination showed a moderate uniform goitre, slight symmetrical exophthalmos, marked tremor of the outstretched hands, a regular tachycardia, a blood-pressure of 120/45, and a hyperkinetic heart, but no evidence of heart failure. The basal metabolic rate was 77 per cent. above normal. The electrocardiogram, chest radiograph, and blood count were normal. The patient also complained that for the past three months her knees had felt weak, and she had had difficulty in rising from the bending or kneeling position without using her hands. There was symmetrical weakness and wasting of the quadriceps and deltoids, but no fibrillation and no alteration in the reflexes, and the sensory system and cranial nerves were intact. The serum electrolytes, calcium, and phosphorus were normal. After a period of rest in bed, sedation, and Lugol's iodine, partial thyroidectomy (Professor A. L. d'Abreu) was performed. The gland showed the usual histological features of thyrotoxicosis. No muscle biopsy was done. After operation she developed evidence of mild tetany, which disappeared within a month with oral calcium. Ten weeks after operation all evidence of myopathy had disappeared, but she was grossly myxoedematous, and has since required 120 mg. of thyroid daily to maintain a euthyroid state.

*Case 4.* A married woman aged 40, employed as a part-time school secretary, was first seen at the General Hospital, Birmingham, on 19.2.60. She had had no previous illness, and had one child aged 12 years. In June 1959 she had noticed tremulousness and loss of weight, and had consulted her doctor, who had prescribed neomercazole and Lugol's iodine. For a time she improved, but at Christmas she noticed that her eyes were puffy, she became dyspnoeic on exertion, and her weight began to fall once more. She was very thyrotoxic, with a moderate uniform goitre, marked tremor of the outstretched hands, bilateral exophthalmos of moderate degree with periorbital oedema, visible arterial pulsation in the neck, a regular tachycardia of 108 per minute, and a hyperkinetic heart. There was slight oedema of the legs, but no other evidence of cardiac failure. The central nervous system was normal. The neomercazole and Lugol's iodine were discontinued, she was given phenobarbitone, and arrangements were made to admit her for surgical treatment six weeks later. On admission she had lost a good deal more weight, and was violently thyrotoxic, the whole bed shaking as she lay in it. She was sweating profusely, was tearful, emotional, and nauseated, and became faint and giddy on sitting up. There was oedema of both legs, and increased jugular venous pressure. The pulse was 132 per minute but still regular, and the blood-pressure 130/70. An attempt was made to estimate the basal metabolic rate, but the patient's very severely thyrotoxic state made it impossible. On examination of the central nervous system all muscle power was weak, especially flexion of the neck, trunk, and thighs. She was unable to get up from lying or kneeling without the assistance of her hands, and had great difficulty in ascending stairs. The thighs, deltoids, and the interossei of the upper limbs were wasted. No fibrillation was present; there was no alteration in the reflexes and no sensory loss, and the cranial nerves were intact. An injection of 2 mg. of prostigmine produced no improvement in the muscular weakness. The serum electrolytes, calcium, and phosphorus were normal, as were the electrocardiogram, the chest radiograph, and blood count. The maximum 24-hour urinary creatine was 177 mg., with a corresponding creatininuria of 1,103 mg. After a period of rest in bed, sedation, and Lugol's iodine, partial

thyroidectomy and left rectus femoris biopsy were carried out (Mr. Victor Brookes). The muscle biopsy showed cellular aggregates of lymphocytes and histiocytes between muscle fibres (Plate 28, Fig. 4), while the thyroid showed the usual appearances of thyrotoxicosis. After operation the patient was given physiotherapy and 25 mg. of nandrolone weekly by injection. Three months later she was euthyroid, and all evidence of myopathy had disappeared.

*Case 5.* A manager of a timber yard, aged 59, was first seen at the General Hospital, Birmingham, in January 1959. In the previous seven months he had lost two stone in weight, and his legs had become weak so that he had difficulty in walking and had fallen on several occasions. There had also been some pain in both legs. Both thighs were weak and wasted, especially the right; the right knee jerk was absent, and there was some hypalgesia of the right leg. The central nervous system was otherwise normal, apart from medullated nerve fibres in both fundi. Sugar was present in the urine, and the blood sugar was 245 mg. per 100 ml. The condition was thought to be a diabetic neuropathy, and the patient was given a 150 g. carbohydrate diet, 0·5 g. tolbutamide three times a day, and vitamin B, and arrangements were made to admit him to hospital at a later date. When he was admitted to the Queen Elizabeth Hospital, Birmingham, a little over a month later, all glycosuria had disappeared, and the blood sugar was 149 mg. per 100 ml., but he had lost a further stone in weight, and his neurological disability had increased. He was unable to get up from lying or kneeling without the assistance of his hands. Neck, trunk, and thigh flexion were very weak, as were flexion and extension of the knees and elbows and dorsiflexion of the feet; both thighs and buttocks were very weak and wasted, and fibrillation was evident. The interossei of the hands were also wasted. The knee and ankle jerks were absent, and there was diminished vibration sense in the right leg, but no other sensory loss could be detected. The cranial nerves were normal. The muscle weakness showed no response to an injection of 2 mg. of prostigmine. It was eventually appreciated that the patient was severely thyrotoxic. Though there was no goitre, symmetrical exophthalmos was present, and there was marked tremor of the outstretched hands and a presistent regular tachycardia. The basal metabolic rate was 71 per cent. above normal. A lumbar puncture showed normal chemistry, cytology, and hydrodynamics of the spinal fluid. X-rays of the chest and spine were normal, the blood count showed no abnormality, and the Wassermann reaction was negative in the blood and spinal fluid. The liver function tests, acid and alkaline phosphatase, serum calcium and phosphorus, blood urea, and serum electrolytes were all normal. The electrocardiogram was normal. The 24-hour urinary excretion of creatine was 175 mg., and the creatinine excretion 1,520 mg. There were no porphyrins in the urine. Despite a free diet, 1·5 g. of tolbutamide daily sufficed to keep the urine free from sugar. Methyl thiouracil was commenced in a dose of 600 mg. daily, and when the patient was euthyroid it was reduced to a maintenance dose of 200 mg. daily. As soon as the thyrotoxicosis was under control his neurological disability began to improve, but before it did so weakness and wasting of the deltoids and upper arms developed. Discontinuance of the tolbutamide in the euthyroid state led to a return of glycosuria and a diabetic glucose-tolerance curve. He has since continued to receive 1·5 g. of tolbutamide daily, and there has been no return of glycosuria. In due course a dose of 50 mg. of methyl thiouracil daily sufficed to maintain normal thyroid function. Fifteen months after the institution of thiouracil therapy the patient, though much improved, still cannot get up from lying or kneeling without the assistance of his hands; the legs are still weak, the interossei of the left hand are still wasted,

FIG. 2. Case 1. Muscle biopsy from the rectus femoris, showing fatty replacement of muscle-fibres (Sudan, ×150)

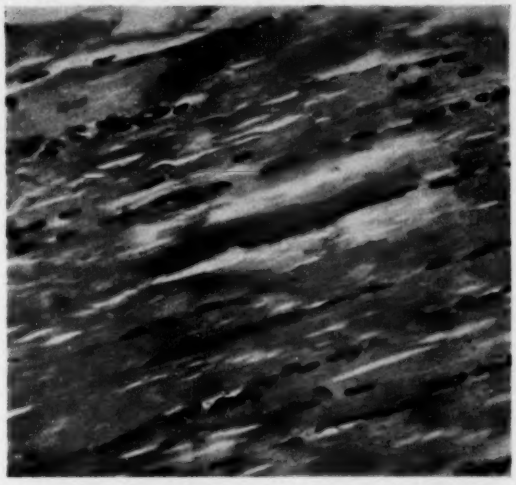

FIG. 3. Case 2. Muscle biopsy from the quadriceps, showing proliferation of sarcolemmal nuclei (haematoxylin and eosin, ×250)

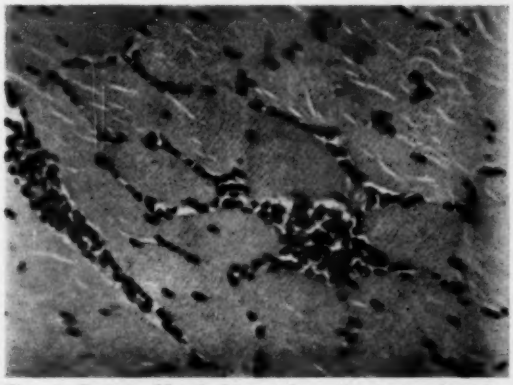

FIG. 4. Case 4. Muscle biopsy from the rectus femoris, showing cellular aggregates consisting of lymphocytes and histiocytes between muscle-fibres (haematoxylin and eosin, ×250)

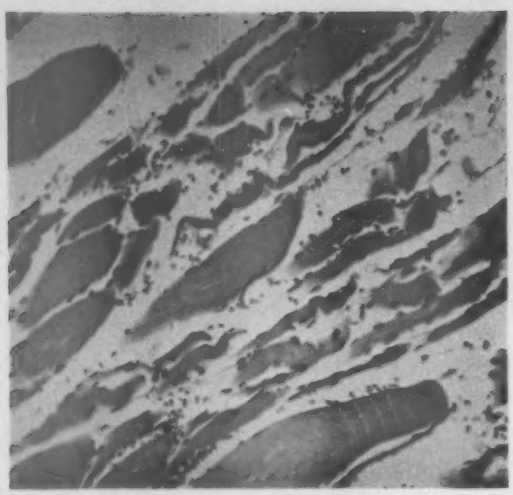

FIG. 5. Case 5. Muscle biopsy from the rectus abdominis, showing atrophy of muscle-fibres (haematoxylin and eosin, ×150)

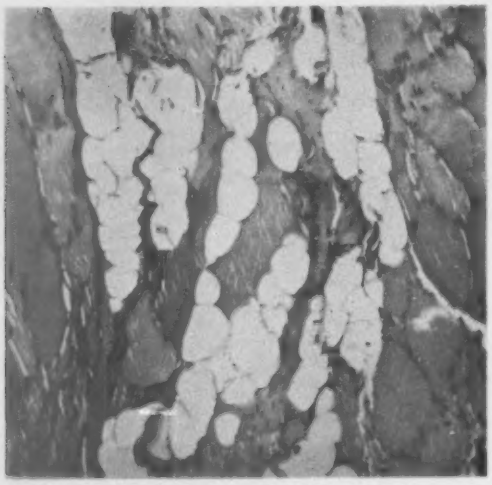

FIG. 6. Case 5. Muscle biopsy from the rectus abdominis, showing fatty replacement of muscle-fibres (haematoxylin and eosin, ×125)

and the left ankle jerk is still absent, though the other tendon jerks in the legs have returned. In May and June 1959 he had severe abdominal pains which defied clinical and radiological diagnosis. A laparotomy was thought necessary, but nothing abnormal was found, and thereafter the abdominal symptoms subsided. A biopsy specimen from the rectus abdominis at this operation showed atrophy and fatty replacement of muscle fibres (Plate 29, Figs. 5 and 6).

We are greatly indebted to Dr. Walter Smith for the histological examination of the muscle biopsy specimens and for the microphotographs, to Dr. Michael Jefferson for the electromyography in Case 1, and to Mr. D. M. Morrissey, Mr. Victor Brookes, and Professor A. L. d'Abreu, who performed the operations.

*Summary*

Five cases of chronic thyrotoxic myopathy are described, and the previously reported cases are reviewed.

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# HYPOGLYCAEMIA AND ABNORMAL STEROID METABOLISM IN ADRENAL TUMOURS[1]

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With Plates 30 and 31

FROM time to time cases of adrenal tumour have been described (Anderson, 1930; Lawrence, 1937; Broster and Patterson, 1948; Staffieri, Cames, and Cid, 1949; Thannhauser, 1949; Dohan, Rose, Eiman, Richardson, and Zintel, 1953; Schamaun, Deucher, and Gablinger, 1957; Aszkanazy, Jenkins, and Simpson, 1958; Boss, 1959) in which the main or presenting clinical feature was hypoglycaemia, despite the production in several instances of increased amounts of corticosteroids. It is of considerable interest that the patient described by Dohan and his colleagues was later found to show an unusual pattern of steroid excretion in the urine, characterized by the presence of large amounts of tetrahydro-S (Touchstone, Richardson, Bulaschenko, Landolt, and Dohan, 1954). This is the main metabolite of 11-deoxycortisol or Reichstein's compound S, one of the naturally occurring precursors of cortisol: normally it is rapidly converted to cortisol in the adrenal, only small amounts being found in adrenal vein blood (Touchstone, 1958). It seemed likely, therefore, that compound S was being secreted in excess by the tumour as the result of a deficiency of the enzyme 11 $\beta$-hydroxylase, which is responsible for the conversion of compound S to cortisol. A similar pattern of urinary steroid excretion is seen after the administration of the 11 $\beta$-hydroxylase inhibitor SU-4885 (Liddle, Island, Lance, and Harris, 1958). The significance of this defect, particularly in relation to the clinical picture, is, however, by no means clear, since it has been demonstrated in other cases of adrenal tumour and hyperplasia in which the main features have been those of Cushing's syndrome (Rosselet, Overland, Jailer, and Lieberman, 1954; Ungar and Dorfman, 1956; Touchstone, Bulaschenko, Richardson, and Dohan, 1957). The two cases described by Rosselet and his colleagues also showed gross oedema, which they thought was due to the excessive secretion of compound S. The same disturbance of steroid biosynthesis has also been found (Eberlein and Bongiovanni, 1955) in the hypertensive form of congenital adrenal virilism.

[1] Received December 1, 1960.

The purpose of the present paper is to describe three further cases of adrenal tumour showing this unusual pattern of steroid excretion, in two of which the main clinical feature was hypoglycaemia. Cases 1 and 2 have already been reported briefly (Williams, 1960).

### *Case Reports*

*Case 1* (Middlesex Hospital No. H. 96045). An unmarried female clerk, aged 24 years, was admitted to The Middlesex Hospital in November 1955, complaining of swelling of the feet and ankles, irregular scanty periods, acne, and

TABLE I

*Electrolyte Values and Renal Function Before and Three Weeks After Removal of the Tumour in Case 1*

| | *Before operation* | *After operation* |
|---|---|---|
| Plasma Na | 146 m-equiv./l. | 138 m-equiv./l. |
| „ K | 2·8 „ | 4·7 „ |
| „ Cl | 107 „ | 95 „ |
| „ $CO_2$ | 28 „ | 25 „ |
| Total exchangeable K | 1,880 m-equiv. | 2,180 m-equiv. |
| Salivary Na/K ratio (corr. to 2 ml./min.) | 0·16 | 0·58 |
| $C_{Cr}$ | 90 ml./min. | 95 ml./min. |
| Maximum s.g. | 1,012 | 1,022 (later 1,027) |
| Minimum pH | 7·0 | 4·8 |
| Water elimination (20 ml./kg.) | — | 1,037 ml. in 3 hrs. |

$C_{Cr}$ = endogenous creatinine clearance; Maximum s.g. = specific gravity of urine 13 hours after intramuscular pitressin tannate, 5 units in oil; minimum pH = urinary pH after 10 g. ammonium chloride.

growth of dark hair on the face, trunk, and limbs. The symptoms had been present for four months.

*On examination* mild virilism was noted, with rather coarse features and small breasts. Dark fine hairs were present on the upper lip, arms, legs, and dorsum of the hands. Dense pubic hair extended up to the umbilicus. The clitoris was not enlarged. Very slight oedema of the ankles was present. The blood-pressure was 220/130; the fundi were normal. A large mass occupied the left upper abdomen. The liver could not be felt.

*Investigations.* The haemoglobin was 91 per cent., the white-cell count 11,000 per cu. mm. (polymorphs 80 per cent., lymphocytes 13 per cent., monocytes 7 per cent.), and eosinophil count 18 per cu. mm. The serum electrolytes showed a hypokalaemic alkalosis, with the serum potassium 2·8 m-equiv. per litre (Table I). The plasma calcium was 9·0 mg. per 100 ml., and the inorganic phosphate 2·4 mg. per 100 ml. The urine contained no albumin or sugar, and the centrifuged deposit a few hyaline casts only. A culture yielded *E. coli*. Renal function tests showed normal blood urea (18 mg. per 100 ml.), slightly reduced creatinine clearance, and marked impairment of tubular function, with inability to concentrate or acidify (Table I). There was good excretion on the intravenous pyelogram; the left kidney was displaced downwards by a mass. The result of an oral glucose tolerance test is given in Table II, and the urinary steroid values in Table III. Not shown in the latter Table are the values for free cortisol (367 μg. per 24 hours; normal 9·5 to 81·5 μg.), free corticosterone (33·6 μg. per 24 hours; normal 2·2 to 9·0 μg.), pregnanetriol (0·9 mg. per 24

hours; normal 0·1 to 1·9 mg.), and pregnanediol (10 mg. per 24 hours; normal 0·2 to 0·7 mg.).

*Progress.* She complained of headache and diplopia in the mornings before breakfast, and on one occasion after fasting for 12 hours became drowsy and disorientated and sweated profusely, these symptoms being promptly relieved by oral glucose. Mild tetany was present intermittently, and heavy vaginal bleeding occurred on two occasions.

*Operation.* On 5.12.55 Mr. C. J. B. Murray removed from the left suprarenal region a large encapsulated tumour, triangular in shape, measuring 17·5 × 12·5 × 7·5 cm. and weighing 2 kg. No metastases were seen. Histological examination (Dr. A. D. Thompson) showed a carcinoma of adrenal cortical tissue,

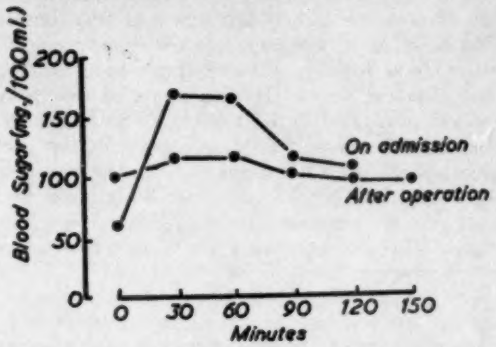


FIG. 1. Glucose tolerance test in Case 1 before and after operation.

with invasion of the capsule. A biopsy from the left kidney showed vacuolar degeneration of the tubules, the most marked changes on microdissection being in the distal parts of the proximal convoluted tubules (Dr. E. M. Darmady).

*Subsequent course.* After the operation tetany and hypoglycaemic symptoms disappeared. The blood-pressure fell to 120/80. Three weeks after operation the blood eosinophils were 156 per cu. mm., the serum electrolytes had returned to normal, and there had been considerable improvement in renal tubular function (Table I). A glucose tolerance test showed a higher fasting level (Fig. 1). Urinary steroid excretion fell to normal levels, menstruation became normal, and the acne and coarsening of the features regressed. The patient remained well for nearly nine months, and then developed metastases, and with this a return of hypoglycaemia. When readmitted in January 1957 she was deeply comatose. She recovered promptly with intravenous glucose but, in spite of glucose 10 g. hourly by mouth, continued to have hypoglycaemic attacks with blood-sugar levels of 40 to 45 mg. per 100 ml., and glucose had to be infused continuously. Cortisone, 25 mg. every six hours, produced no improvement. Urinary steroid estimations again showed very high levels (Table III). Plasma insulin-like activity was measured by Dr. Vallance-Owen using the rat diaphragm, and was found to be normal. As there appeared to be some response to corticotrophin stimulation (Martin, 1960), pituitary stalk section was carried out by Mr. Valentine Logue, but the patient died shortly afterwards.

*Post mortem* there was massive invasion of the liver by carcinoma, the total mass weighing 9·4 kg. Deposits were also present in the lungs. The pancreas was normal. An extract of tumour tissue was assayed for insulin-like activity

by Dr. K. L. Smith of the Bio-assay Division, Boots Pure Drug Company. No activity was detected.

*Case 2* (Hammersmith Hospital No. 222728). A 48-year-old labourer was admitted to the Hammersmith Hospital in January 1959, with a two-month history of increasing breathlessness on exertion, paroxysmal nocturnal dyspnoea, and swelling of the ankles. On examination he was in congestive cardiac failure, with gross pitting oedema of the legs and a raised jugular venous pressure. The blood-pressure was 220/140. He was also noted to have a red face and to be sweating profusely, though little account was taken of this at the time. The diagnosis was hypertensive heart failure, and he responded well to routine treatment with digitalis, diuretics, and mecamylamine. Three months later, however, he was readmitted, complaining of abdominal discomfort and attacks of trembling; these attacks started in the legs and then became generalized, lasting up to half an hour, and were associated with a feeling of weakness.

*On examination* the facial flushing and sweating was much more marked, and extended to all the exposed areas. It was more or less persistent, though aggravated by emotion and possibly by fasting. The rest of the body was of normal colour. The liver was grossly enlarged and irregular, extending almost to the pelvis. The other abnormal findings were in the nervous system: there was diffuse weakness and wasting in the upper limbs, and proximally in the lower limbs, with occasional fasciculation. All reflexes were sluggish, but there were no sensory signs. The blood-pressure was well controlled at 150/100, and the venous pressure was normal.

*Investigations.* The haemoglobin was 110 per cent., white-cell count 12,000 per cu. mm., platelets 259,000 per cu. mm., and reticulocyte count 4·8 per cent. The sternal marrow showed active erythropoiesis consistent with polycythaemia; the red-cell volume was 36 ml. per kg., and blood volume 74 ml. per kg. The serum sodium was 140 m-equiv., potassium 3·8 m-equiv., chloride 95 m-equiv., and bicarbonate 31 m-equiv. per litre. The blood urea was 42 mg. per 100 ml. Excretion of 5-hydroxyindole acetic acid was 4·6 mg. per 24 hours. The serum 5-hydroxytryptamine, the serum and urinary histamine levels, and the urinary catechol amines, were all within normal limits. The results of the urinary steroid analyses are given in Table III.

*Liver function tests* showed serum bilirubin 0·5 mg. per 100 ml., thymol turbidity 2 units, alkaline phosphatase 11 King–Armstrong units, and serum transaminases G-OT 31 units, G-PT 29 units. The serum cholesterol was 105 mg. per 100 ml., and the prothrombin time normal. The serum albumin was 2·2 g. and globulin 2·9 g. per 100 ml., an electrophoretic strip showing raised $\alpha_2$ and $\beta$ peaks. The liver biopsy specimen consisted entirely of tumour tissue, the appearances being those of an adrenocortical tumour. The tumour cells were large and polygonal, with mixed granular and foamy cytoplasm, and contained glycogen. The nuclei varied considerably in size and shape, and mitoses both normal and abnormal were numerous.

*Carbohydrate metabolism.* The fasting blood sugar on 2.6.59 was 37 mg. per 100 ml. The glucose tolerance test done on 12.6.59 is illustrated in Fig. 2. The fasting level on this occasion was 31 mg. per 100 ml., and after 50 g. glucose by mouth the maximum rise, to 97 mg. per 100 ml., was at one hour. The test was continued after the two-hour period, and by four hours the level had fallen to 25 mg. per 100 ml., at which it appeared to become stabilized. The patient was uncomfortable and sweating at this stage, but fully conscious and orientated. At six hours he was given 1·5 ml. of 1:1,000 adrenaline subcutaneously, and the blood sugar increased slowly to 57 mg. per 100 ml. during the next two hours (Fig. 2). There was no significant change in the level of serum inorganic

phosphate throughout the test. The insulin sensitivity test showed an initial fasting level of 50 mg. per 100 ml., the maximum fall to 23 mg. per 100 ml. occurring 30 minutes after the soluble insulin (7 units). The 45- and 60-minute samples showed similar levels, but by two hours the blood sugar had risen to 41 mg. per 100 ml. The serum insulin-like activity was normal (Dr. P. Wright). A second liver biopsy specimen, taken directly into carbon dioxide snow, showed absence of glucose-6-phosphatase in the tumour cells (Dr. A. G. E. Pearse).

*Progress.* The patient went steadily downhill, his main complaints being abdominal discomfort and vomiting. The marked flush and sweating continued and, although at times he complained of weakness and faintness, particularly

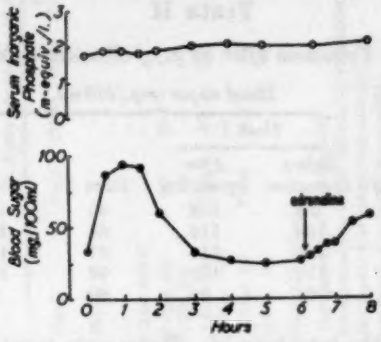


FIG. 2. Extended glucose tolerance test and response to subcutaneous adrenaline in Case 2.

during fasting, there were no definite episodes of hypoglycaemic coma or convulsions. He died on 26.6.59, six weeks after admission.

*Post mortem* there was a massive tumour, weighing 1·1 kg., which replaced the left adrenal and displaced the kidney downwards. A tongue of growth filled the adrenal vein and projected into the inferior vena cava. Massive secondary deposits were present in the liver. These could easily be shelled out, about 3 kg. of the total liver weight of over 5·5 kg. being tumour tissue, and the remainder apparently uninvolved. There were no other secondary growths. Both kidneys were enlarged (220 g. each), but otherwise normal. The heart was enlarged (455 g.) with hypertrophy of both chambers. There was confluent bronchopneumonia of both lungs, and three acute ulcers in the stomach. It was noted at the autopsy that the intense deep purple of the face and neck was greatly decreased after the skin and great veins of the neck had been cut.

Microscopy showed that the primary tumour was an adrenal cortical carcinoma, mostly well differentiated, with a trabecular pattern. In a few areas there were foamy cells, and in other places the tumour was poorly differentiated, with marked nuclear variation in size and staining, and frequent mitoses. There was extensive necrosis. Anaplasia was even more prominent in the secondary deposits. The small right adrenal (3·5 g.) showed a very thin cortex, with the zona reticularis and fasciculata represented by only a narrow layer of foamy cells, while the medulla and zona glomerulosa appeared normal (Plate 30, Fig. 3). The pituitary was of normal size. In the anterior lobe the majority of the basophils showed perinuclear hyalinization of the cytoplasm (Crooke cells; Plate 31, Fig. 5*a*), and the number of vesiculate basophils was also increased (Fig. 5*b*). The acidophils also showed signs of great activity, with groups of

partially or completely degranulated cells. The thyroid contained plentiful colloid, and the follicles were lined by cuboidal epithelium. The small testes showed absence of mature spermatozoa and a great reduction in the number of Leydig cells. The parathyroids were normal. A very severe myopathy was present in a section of one quadriceps muscle. There were no unaffected fibres, and the least affected ones, with loss of cross striation, were mixed at random with moderately and severely affected fibres, which were little more than collapsed skeletons with crowded sarcolemmal nuclei (Plate 30, Fig. 4). No abnormality was found in the brain or spinal cord. Sections of sternum and vertebrae showed a moderate osteoporosis, and there was a virtual absence of

TABLE II

*Glucose Tolerance after 50 g. of Glucose by Mouth*

| | *Blood sugar (mg./100 ml.)* | | | |
|---|---|---|---|---|
| | *Case 1* | | | |
| *Time (minutes)* | *Before operation* | *After operation* | *Case 2* | *Case 3* |
| 0 | 58 | 102 | 31 | 110 |
| 30 | 168 | 114 | 84 | 164 |
| 60 | 164 | 116 | 97 | 144 |
| 90 | 114 | 100 | 93 | 136 |
| 120 | 104 | 94 | 60 | 98 |

germinal centres from the lymph-nodes. There were many dilated blood-filled small veins in the upper dermis of the skin from the neck, and nearly complete absence of melanin from the basal layer of the epidermis.

*Case 3* (Middlesex Hospital No. H. 90207). Massive oedema of the legs, due to obstruction of the inferior vena cava, was the only symptom in this patient, a housewife aged 49. The complexion was florid. There was some virilism, but no Cushing features. Amenorrhoea had been present for three months, and dark hair was noted on the face, arms, and hands. The clitoris was normal. Blood-pressure was 155/80. A mass in the right hypochondrium proved at operation to be a tumour of the right adrenal, with extensive involvement of lymph-nodes around the vena cava. The liver was enlarged, but showed no obvious malignancy. Histologically the tumour was poorly differentiated. Hypoglycaemic symptoms were absent. Random blood-sugar values were 88 and 155 mg. per 100 ml. The glucose tolerance test was normal apart from a slightly raised fasting level (Table II). There was no significant change in the level of serum inorganic phosphate during this test (2·4, 2·3, 2·3, 2·4, 2·3, m-equiv. per litre at 0, 30, 60, 90, and 120 minutes respectively). Urinary steroid values are shown in Table III. Little or no increase occurred after corticotrophin.

### *Urinary Steroid Excretion*

*Methods.* These were minor modifications of established methods. 17-keto-steroids were analysed by gradient elution from alumina adsorption columns (Kellie and Wade, 1957), and 17-hydroxy-corticosteroids by gradient elution from silica partition columns. Determination of 17-ketosteroids was made by the Zimmermann reaction, and that of 17-hydroxy-corticosteroids by the blue tetrazolium reaction (Chen, Wheeler, and Tewell, 1953). The identity of steroids

## TABLE III

### *Urinary Steroid Analyses*

| Case number | 17-Ketosteroids (mg./day) | | | | | | | 17-Hydroxycorticoids (mg./day) | | | | | | Aldosterone (μg./day) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Gluc. | $SO_4$ | D | A | E | 11-oxy-KS | Total | THS | THE | THF | P'triol + 17'OHp'olone | Compound S | |
| 1 | | | | | | | | | | | | | | |
| On admission | 56·8 | 28·5 | 28·2 | 19·9 | 9·1 | 17·0 | 4·8 | 162·0 | .. | .. | .. | .. | .. | 5·8 |
| After cortisone | 34·3 | 20·1 | 14·2 | 8·4 | 5·1 | 13·7 | 2·1 | .. | .. | .. | .. | .. | .. | .. |
| Post-operative | 0·9 | .. | .. | 0·16 | 0·15 | 0·24 | 0·18 | .. | .. | .. | .. | .. | .. | .. |
| With secondary growths | 71·6 | 21·6 | 50·0 | 43·9 | 4·4 | 17·3 | 2·9 | 254–480 after ACTH | .. | .. | .. | .. | .. | .. |
| 2 | 130·1 | 30·4 | 99·7 | 80·8 | 15·2 | 11·3 | 13·9* | > 400 | Gluc. 210<br>$SO_4$ 115 | 147<br>46 | 16·8<br>.. | 6·3<br>.. | 10·3<br>.. | ..<br>+ | Not measured |
| 3 | 70·3 | 20·8 | 39·5 | 28·2 | 10·8 | 19·2 | 7·3* | 69·0 | Gluc. 55·4<br>$SO_4$ 6·6 | 17·1<br>.. | 9·6<br>.. | 17·3<br>.. | Nil<br>.. | ..<br>.. | 12<br>.. |
| Normal range (female: 20–30 yrs.) | 4·8 – 13·7 | 4·1 – 10·9 | 2·80 – 3·2 | 0·96 – 1·40 | 2·67 – 4·07 | 4·10 – 6·20 | 0·46 – 0·91 | 6·9 – 16·0 | Gluc. 8·2<br>$SO_4$ Nil | Trace<br>.. | 1·6<br>.. | 2·8<br>.. | 1·0 – 6·0 | Nil | 4·6 – 18·9 |

* Not all characterised; many other compounds present.

*Abbreviations:* Gluc. = glucuronide.
D = dehydroepiandrosterone.
A = androsterone.
E = aetiocholanolone.

11-oxy-KS = 11-oxygenated 17-ketosteroids.
THS = tetrahydro-S (3α:17α:21-trihydroxy-5β-pregnan-20-one).
THE = tetrahydro-E or tetrahydro-cortisone.
THF = tetrahydro-F or tetrahydro-cortisol.
P'triol = 5β-pregnane-3α:17α:20α-triol.
17'OHp'olone = 3α:17α-dihydroxy-5β-pregnan-20-one.
S = 17α:21-dihydroxy-pregn-4-ene-3:20-dione.

was established by paper chromatography of the free compounds and of acetates (Bush, 1952) and confirmed by infra-red absorption spectra. It is to be noted that initially the urine samples in Case 1 were analysed only for total 17-ketosteroids and 17-hydroxy-corticosteroids, and differential analysis was confined to the former fraction. Subsequently, after examination of urine from the remaining patients had shown an abnormality in the excretion of tetrahydro-S (THS), the composition of the 17-hydroxy-corticosteroid fraction was examined; for this reason the data quoted for Case 1 refer to stored urine samples.

TABLE IV

*Pre-operative Renal Clearance of Ketosteroids in Case 1*

(Urine volume 185 ml. in 120 minutes)

| | *Sulphate* | | | *Glucuronide* | | |
|---|---|---|---|---|---|---|
| | *Urine* μg./100ml. | *Plasma* μg./100ml. | *Clearance* ml./min. | *Urine* μg./100ml. | *Plasma* μg./100ml. | *Clearance* ml./min. |
| Dehydroepiandrosterone (D) | 1,480 | 39·5 | 58 | 55 | (3·2) | .. |
| Androsterone (A) . . | 214 | 6 | 53 | 390 | (1·2) | .. |
| Aetiocholanolone (E) . | 165 | (3·6) | .. | 1,510 | 9·2 | 253 |

Values in parentheses are approximate only.

*Results.* Full details are given in Table III. As the three cases showed a similar pattern of steroid excretion, they are considered together.

*17-ketosteroids.* All show a massive rise of 17-ketosteroids, with a proportionally great increase of sulphate (dehydroepiandrosterone) fraction typical of adrenal carcinoma. The 11-deoxy- and 11-oxy-17-ketosteroids were also increased, and paper chromatography showed the presence of abnormally high amounts of other steroid metabolites which were not characterized. It can be seen from Table III that in Case 1 a definite decrease in ketosteroid output followed the administration of cortisone. The drop to normal following removal of the tumour is also shown, and the subsequent rise to even higher levels with the appearance of metastases. The clearance rates for the 17-ketosteroid conjugates, which were also studied at the time of first admission, were more than five times the normal (Table IV).

*17-hydroxy-corticosteroids.* These were substantially increased in all patients, the highest levels being found in Case 2, where the total 17-hydroxy-corticosteroid excretion exceeded 400 mg. per 24 hours. Break-up of the total figures shows an increase in THS, THE, and THF; the data here are complete only for Cases 2 and 3. Retrospective examination of a urine sample from Case 1 which had been stored at −10° C showed, however, that there was a similar great excess of tetrahydro-S. It is important to note that although this increase in tetrahydro-S was proportionally very much greater than that of THE and THF (it represented, for instance, 70 per cent. of the glucuronide fraction in Case 2), nevertheless the amounts of the latter compounds were substantially greater than normal. The amount of free cortisol in the urine of Case 1 on first admission was measured, and was considerably increased (367 μg. in 24 hours;

normal range 9·5 to 81·5 μg. in 24 hours). The presence of measurable amounts of 17-hydroxy-corticosteroids in the sulphate fraction (Case 2) is abnormal, and the presence of compound S (17α:21-dihydroxy-pregn-4-ene-3:20-dione) in this fraction has not previously been reported. Also present in this urine were excessive amounts of pregnanediol and pregnanetriol; the latter compound, although non-reducing, contributes to the 17-hydroxy-corticosteroid total. It can be seen (Table III) that in Case 1 during the final admission a marked increase in total 17-hydroxy-corticosteroids followed corticotrophin administration; this finding, together with the decrease in 17-ketosteroids following cortisone administration previously referred to, indicates that the neoplastic tissue, even after removal of the primary tumour, was to some extent under the control of the pituitary (Martin, 1960).

## *Discussion*

*Urinary steroid excretion.* A remarkable feature common to all three cases was the presence in the urine of large amounts of tetrahydro-S, up to 193 mg. per 24 hours in Case 2. There seems little doubt that this was the result of excessive secretion of compound S by the tumour. The tetrahydro derivative has been identified as its major ketol metabolite (Richardson, Touchstone, and Dohan, 1955), and normally only small quantities are found in the urine. Touchstone, Bulaschenko, Richardson, and Dohan (1957) found levels up to 0·02 mg. per 24 hours in pooled normal urine. In a case of congenital adrenal hyperplasia with a high urinary tetrahydro-S excretion, Eberlein and Bongiovanni (1955) succeeded in isolating free compound S as well as the tetrahydro derivative from the blood. The free compound, however, has not previously been isolated from urine. Touchstone and his colleagues (1957) were unable to detect it either in the cases of adrenal tumour or in subjects with adrenal insufficiency given compound S by mouth, although the other alpha ketols were invariably found in the urine in appreciable amounts after administration of comparable doses. They concluded that compound S was one of the most actively metabolized of the adrenal ketolic steroids. Its appearance in the urine in Case 2 can be attributed to overloading, or more probably to impairment of reduction systems in the liver. If, as seems likely, the excessive secretion of compound S by these tumours was due to a defect in 11 β-hydroxylation, then clearly this defect was a relative one. The amounts of tetrahydro-E and -F in the urine, although small in comparison with that of tetrahydro-S, were nevertheless increased above the normal range. In Case 1 free cortisol and free corticosterone were measured and found to be increased. Normal amounts of urinary aldosterone, another 11-oxygenated steroid, were found in all three cases. These findings agree with those of Touchstone, Bulaschenko, Richardson, and Dohan (1957), who found an increased excretion of cortisol and its tetrahydro derivative in five out of six cases, although tetrahydro-S accounted for 45 to 92 per cent. of the ketolic metabolites seen in this chromatographic

fraction. In their sixth case the cortisol excretion was normal, and it is of interest that it was this patient who showed hypoglycaemia.

*Relation to the clinical picture.* The most important question to be considered is that of the relation of this disturbance of steroid synthesis to the clinical picture. We have set out in Table V the clinical features described in previously reported cases of adrenal hyperplasia or tumour (excluding the congenital variety) in which large amounts of tetrahydro-S have been found in the urine.

TABLE V

*Previously Reported Cases of Adrenocortical Tumour and Hyperplasia with Similar Pattern of Steroid Excretion*

| *Cases reported by* | *Pathological diagnosis* | *Clinical picture* | *Excretion of* | |
|---|---|---|---|---|
| | | | *THS* | *THE and THF* (*mg./24 hours*) |
| Rosselet, Overland, Jailer, and Lieberman (1954) | Carcinoma | Both had Cushing's syndrome with excessive oedema | 11·2 | 16 |
| | Carcinoma | | 3·4 | 4 (1–6) |
| Ungar and Dorfman (1956) | Carcinoma | Cushing's syndrome | .. | .. |
| Touchstone, Bulaschenko, Richardson, and Dohan (1957) | Hyperplasia | Cushing's syndrome | 0·25 | 2·5 |
| | Hyperplasia | ,, ,, | 1·7 | 5·3 |
| | Carcinoma | ,, ,, | 15·0 | 5·0 |
| | Carcinoma | Feminism | 2·5 | 2·7 |
| | Carcinoma | Virilism (and hypoglycaemia) | 12·4 (0.02) | 1·1 (1·3) |
| Balls, Nicholson, Goodman, and Touchstone (1959) | Adrenal hyperplasia associated with functioning islet cell carcinoma | Cushing's syndrome | 11·6 (0·05) | 25·6 (6·4) |

The normal values given by the author for the steroid estimations are shown in parentheses.

*Abbreviations*: THS = tetrahydro-S(3α:17α:21-trihydroxy-5β-pregnan-20-one).
THE = tetrahydro-E *or* tetrahydro-cortisone.
THF = tetrahydro-F *or* tetrahydro-cortisol.

It can be seen that in the majority the picture has been that of Cushing's syndrome, though in the patients of Rosselet, Overland, Jailer, and Lieberman (1954) this was complicated by excessive oedema. The series of Touchstone, Bulaschenko, Richardson, and Dohan (1957) included a feminizing adrenal tumour and one with virilism, the patient with the latter condition also having severe and intractable hypoglycaemia. The present cases presented neither the classical picture of Cushing's syndrome nor that of adrenal virilism. The first patient had hypertension and hypokalaemic alkalosis, but looked normal except for mild hirsutism. The second patient had hypertension and mild polycythaemia, but the appearance was certainly not that of Cushing's syndrome, and the diagnosis of adrenal tumour for long remained unsuspected. Hypoglycaemia was present in both cases. The only apparent endocrine abnormalities in the third patient were very slight hirsutism and amenorrhoea. The question whether any of these features could be attributed to the excessive secretion of

compound S is complicated by the fact that cortisol (and in Case 1 corticosterone) production was also increased, though to a relatively small extent. Compound S is usually regarded as physiologically inert, but there is some evidence that it may have weak glucocorticoid activity. Thus Verzár (1952) showed that it could increase glycogen breakdown in the isolated rat diaphragm, and Bergenstal, Huggins, and Dao (1955) found that, in intramuscular doses of 25 to 50 mg., it would maintain life in man after adrenalectomy, though with borderline insufficiency. To induce changes in sodium and chloride balance in man at least 200 mg. per day must be given (Thorn, Laidlaw, and Goldfien, 1955).

It is perhaps surprising, if compound S does have glucocorticoid activity, that our second patient, who had the highest levels of tetrahydro-S in the urine (193 mg. per 24 hours), showed so few signs of Cushing's syndrome during life. It is to be noted, however, that post-mortem examination showed osteoporosis and suppression of germinal centres in the lymph-nodes, which are well-known manifestations of excess of circulating cortisol. The changes in the muscle, with marked necrosis, were similar to, though more severe than, those found in triamcinolone myopathy (Williams, 1959), and also closely resembled those seen experimentally in rabbits given large doses of cortisone (Ellis, 1956); it seems likely that this was another example of a steroid-induced myopathy. The osteoporosis and other changes were all conspicuous, and appeared to be out of proportion to the observed levels of cortisol metabolites in the urine. The opposite adrenal was atrophic, indicating suppression of corticotrophin production. Cortisol is usually regarded as the only steroid produced by the normal adrenal which affects corticotrophin production by the pituitary (Bongiovanni and Eberlein, 1955). The current use of SU-4885 to test pituitary reserve also depends on the assumption that there is no suppression of corticotrophin production by compound S. On the other hand, Tuchmann-Duplessis (1952) found that in rats changes in the pituitary following administration of compound S were very similar to those seen to follow cortisone or corticosterone. So enormous were the quantities of compound S produced by the tumour in Case 2 that some effect on the pituitary could well be expected. Microscopically the basophils showed marked hyaline change. This change was also found *post mortem* in a patient with hypoglycaemia and adrenocortical tumour reported by Boss (1959), who similarly showed no signs of Cushing's syndrome during life. The other findings in the pituitary in Case 2 are more open to speculation; for instance, the vesiculate basophils were increased. These cells are thought to be concerned with thyrotrophin production (Ezrin, Swanson, Humphrey, Dawson, and Hill, 1959) and, as it has been suggested that cortisone can interfere with thyroxine production (Beck, 1958), such an action might account for the changes in the present case. A similar increase was noted by Pearse (1953) in a case of Cushing's syndrome, and also in a patient with adrenal virilism. The degranulation seen in the acidophile cells suggests increased production of growth hormone, which could perhaps be correlated with the presence of splanchnomegaly. It is of interest here that in one of the previously reported

cases of adrenocortical carcinoma and hypoglycaemia (Aszkanazy, Jenkins, and Simpson, 1958) large internal organs were also described. Mention must be made of the striking, bright-red flush of the face and exposed areas which this patient showed. This was quite unlike the plethora seen in Cushing's syndrome. Although the immediate cause was dilatation of the vessels, as confirmed *post*

TABLE VI

*Previously Reported Cases of Hypoglycaemia and Adrenocortical Tumour*

| *Case reported by* | *Age (years) and sex* | *Symptoms apart from hypoglycaemia* | *Other findings* | *Steroid assays* | *Autopsy findings* |
|---|---|---|---|---|---|
| Anderson (1930) | 33 M | None | B.P. 95/80. Hypoglycaemia did not respond to subcutaneous adrenaline | — | Encapsulated tumour of L. adrenal (400 g.); other adrenal small and fibrous. No metastases |
| Lawrence (1937) | 24 F | None | B.P. 100/120. Some response to adrenaline. Tumour of L. adrenal size of large grape-fruit | — | No autopsy performed |
| Broster and Patterson (1948) | 14 F | Virilism | B.P. 120/85 | 17-ketosteroids 1,980 mg./day | Tumour of R. adrenal (2·98 kg.). No metastases |
| Staffieri, Camus, and Cid (1949) | 25 M | Gynaecomastia | Thyroid enlargement. Hepatosplenomegaly. Tumour (sarcoma) measured 20 × 9 × 11 cm. | 17-ketosteroids 23 mg./day | — (Well two years after removal) |
| Dohan, Rose, Eiman, Richardson, and Zintel (1953) | 48 F | Virilism Muscle weakness | B.P. 176/106. Hypokalaemia. Tumour approx. 2·2 kg. | Oestrogens. 17 ketosteroids and 17-hydroxycorticoids increased | Widespread metastases. Remaining adrenal (R.) weighed 7 g. |
| Schamaun, Deucher, and Gablinger (1957) | 37 M | Portal and inferior venacaval compression | Tumour (adenoma) of L. adrenal weighing 1.7 kg. | 17 ketosteroids 35–41 mg./day | — (Well 6 months after removal) |
| Aszkanazy, Jenkins, and Simpson (1958) | 26 F | Abdominal pain | B.P. 130/70 | — | Carcinoma L. adrenal (2.2 kg.); multiple metastases. Extramedullary haemopoiesis. Pituitary and other adrenal normal |
| Ross (1959) | 64 M | None | B.P. 170/80 | — | Carcinoma L. adrenal; metastases in liver; 3 peptic ulcers. Pituitary showed Crooke cells |

*Note.* This Table does not include the report by Thannhauser (1940) of three cases of adrenal carcinoma and hypoglycaemia, in which only brief clinical details are given.

*mortem*, the underlying mechanism is quite unexplained. The surprising lack of melanin that was noted in skin taken from an exposed area may be of importance here. Possibly this was related to suppression of corticotrophin and melanophore-stimulating hormone (MSH) by the increased corticoid production. The release of MSH can be inhibited by cortisone (Lerner, Shizume, and Bunding, 1954).

Another clinical feature of interest is the hypertension which was present in the first two cases. Eberlein and Bongiovanni (1955) regarded this as the distinguishing feature of the type of congenital adrenal hyperplasia in which there is a defect in 11 $\beta$-hydroxylation. In the more common variety, in which the block occurs earlier in the biosynthetic pathway, the blood-pressure is normal. They attributed the hypertension to excessive secretion of deoxycorticosterone, though it is to be noted that hypertension has not been found in all the reported cases (Gandy and Keutmann, 1958). In the present cases the

hypertension certainly appeared to be of recent onset, and in the first patient the blood-pressure dropped to normal on removal of the tumour. In neither patient, however, could an excess of deoxycorticosterone or its metabolites be detected in the urine.

*Mechanism of hypoglycaemia.* There can be little doubt that the hypoglycaemia in the first two cases was due to the adrenal tumour. It disappeared in the first patient after removal of the growth, and reappeared with the development of metastases. In two previously reported cases (Table VI) the tumour was also removed surgically, with similar relief of hypoglycaemia. Why the tumour caused hypoglycaemia is, however, by no means clear. There are several possibilities. The first is that the hypoglycaemia is related to the abnormal steroid metabolism, and in particular to the block in 11 $\beta$-hydroxylation, with resulting defective synthesis of cortisol. This has been the explanation put forward for the hypoglycaemia occasionally seen in cases of congenital adrenal hyperplasia, in which it is sometimes induced by stress (White and Sutton, 1951; Wilkins, Crigler, Silverman, Gardner, and Migeon, 1952; Conn and Seltzer, 1955). Ely, Bray, Raile, and Kelley (1954) found, indeed, that both the fasting blood sugar and the plasma 17-hydroxycorticoid concentration were significantly reduced in patients with congenital adrenal hyperplasia compared with normal children. Yet this can hardly be the explanation in the present cases, for, although there was a similar block in the metabolic pathway, we have already seen that this was not absolute, and there was evidence of increased rather than decreased cortisol production. It can be seen from Table VI that none of the previously reported cases showed any clinical evidence of adrenal insufficiency, though the urine was examined by chromatography in only one patient (Dohan, Rose, Eiman, Richardson, and Zintel, 1953). In this patient the excretion of cortisol metabolites was within the normal range. It seems an extraordinary coincidence that this latter patient and our Cases 1 and 2 showed the same unusual pattern of steroid excretion. One wonders also whether this was not present in some of the earlier cases reported, for none of them was described as showing the classical picture of Cushing's syndrome or adrenal virilism, and in three the blood-pressure was raised. The fact is to be stressed, however, as we have already seen, that other cases of adrenal tumour, including our Case 3, may exhibit the same defect in steroid synthesis without hypoglycaemia. The possibility that the hypoglycaemia may be due not to lack of cortisol, but to excess of compound S, must also be considered. Against this is the evidence we have already quoted that compound S may well have some cortisol-like activity, particularly at the levels found in the present cases. Moreover, we have been unable to lower the blood-sugar level with compound S in a patient with Cushing's syndrome, and Slater (1960) could find no hypoglycaemic effect in rats. It may be suggested that the hypoglycaemia is due to hepatic insufficiency. This cannot, however, be the explanation in our patients, for in both cases the liver function tests were normal during life, and in the first case hypoglycaemia occurred at a time when there were no macroscopic secondary growths in the liver.

The difficulty of explaining hypoglycaemia in these patients leads to the broader problem of why it occasionally occurs in other non-pancreatic tumours such as retro-peritoneal fibromas, fibrosarcomas, and primary hepatomas. In many such cases hypoglycaemia, as in our patient, disappeared with removal of the tumour, and recurred when metastases appeared. It has been suggested (Skillern, McCormack, Hewlett, and Grile, 1954) that these tumours secrete an insulin-like substance. The evidence here is conflicting. August and Hiatt (1958), in a patient with an intrathoracic fibrosarcoma, demonstrated a considerable increase in insulin-like activity in the tumour tissue, and calculated that it was equivalent to three times the activity present in the pancreas of a normal woman. They pointed out, however, that this was a measure of activity rather than of insulin itself, and the results may simply reflect greater metabolic activity with increased carbohydrate utilization. Other investigators (Howard, 1955; Scholz, Woolner, and Priestley, 1957; Sellman, Perkoff, Null, Kimmel, and Tyler, 1959) who have studied non-pancreatic tumours with hypoglycaemia have found insulin-like activity within normal limits. It was also normal in the present cases, both in the plasma during life and, in Case 1, in the tumour tissue examined *post mortem*.

It seems more likely that hypoglycaemia in all such patients has a common mechanism in excessive glucose consumption by the tumour. There is general agreement that neoplastic tissues exhibit a high rate of glycolysis, and a feature of all the reported cases, including the adrenal carcinomas, has been the massive size of the tumour. It has also been shown (McFadzean and Yeung, 1956; Klein and Klein, 1959) that in patients with primary hepatomas with hypoglycaemia there is little change in the level of serum inorganic phosphate, as in our second patient, during the glucose tolerance test. Normally there is a fall, which has been shown (Forsham and Thorn, 1949) to be a valid index of peripheral utilization of glucose. The absence of such a fall suggests that glucose is being diverted from the periphery to the tumour. It is of interest also in connexion with the present cases that McFadzean and Yeung (1956) found a diminished hyperglycaemic response to subcutaneous administration of adrenaline, despite the presence of significant quantities of glycogen in the tumours. It was later suggested that this was because the tumours lacked glucose-6-phosphatase, and this was indeed demonstrated in our second patient, who also showed a slow response to adrenaline. Sellman, Perkoff, Null, Kimmel, and Tyler (1959) found high levels of glycogen and amino acids in a mesothelial-cell sarcoma, suggesting that the tumour was actively synthesizing or concentrating amino acids and using and storing carbohydrate. Possibly the mechanism may vary from case to case. In a preliminary report of a patient with leiomyosarcoma, Butterfield, Kinder, and Mahler (1960) suggested that the hypoglycaemia was due to inhibition of the hepatic output of glucose. They found no change in the specific activity of plasma glucose during a period of 60 minutes after the injection of radioactive glucose; in other words, there was no entry of unlabelled glucose into the blood during this time. They also observed that at operation there was virtually no difference in the glucose levels between blood from an artery and blood from a vein draining the tumour.

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*Summary*

1. Three cases of adrenocortical carcinoma showing an unusual pattern of urinary steroid excretion are described.

2. In each case the principal urinary 17-hydroxy-corticosteroid was tetrahydro-S, although increased amounts of cortisone and cortisol metabolites were also found.

3. These patients did not show the classical picture of Cushing's syndrome or adrenal virilism, and in two the main clinical feature was hypoglycaemia.

4. Reasons are given for believing that the hypoglycaemia was not related to the abnormal steroid production.

5. It is suggested that the mechanism of hypoglycaemia in these cases, and in other large non-pancreatic tumours, may be excessive uptake of glucose by the tumour mass.

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FIG. 3. Right adrenal in Case 2, with normal medulla and greatly narrowed cortex (haematoxylin and eosin, ×40)

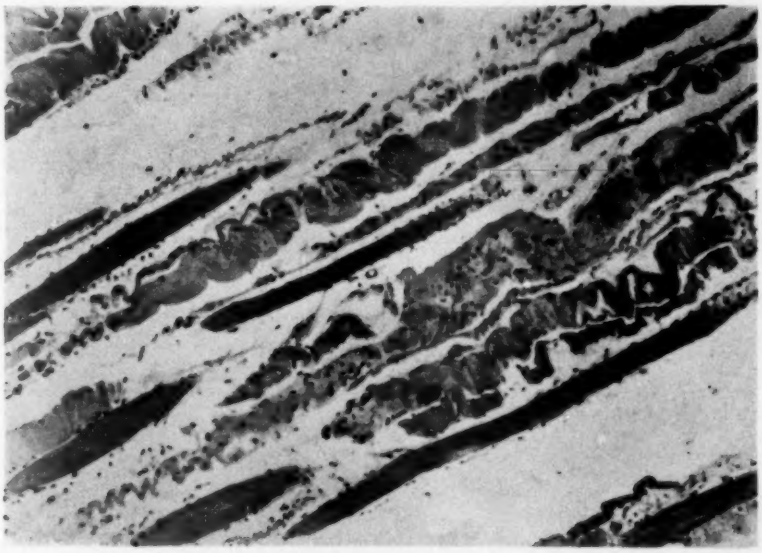
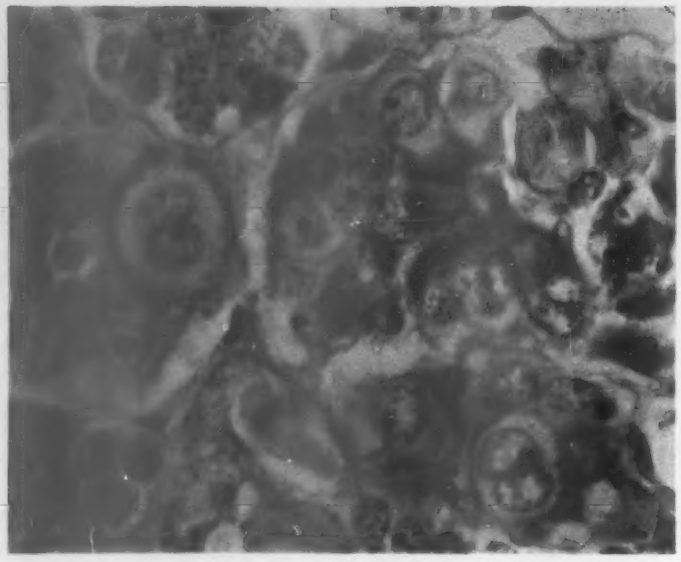
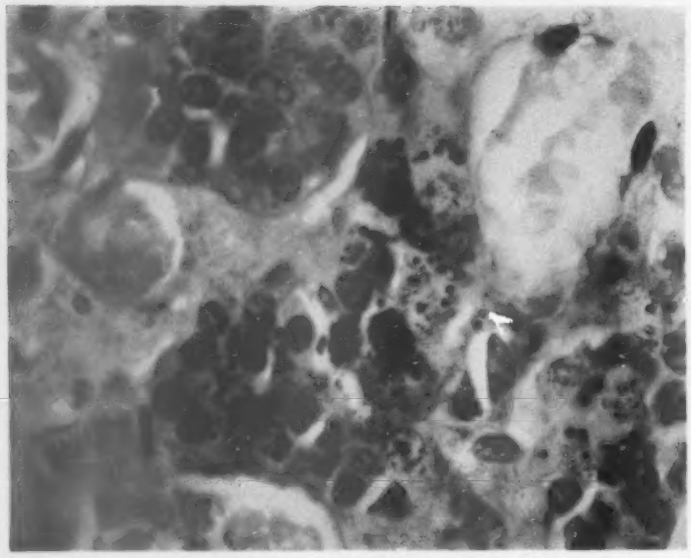


FIG. 4. Section of muscle showing severe myopathy (haematoxylin and eosin, ×100)



*a*



*b*

FIG. 5. Pituitary showing (*a*) Crooke cells, (*b*) vesiculate basophils (tripas, × 1,000)

616.455

# FUNCTIONAL RELATIONSHIP BETWEEN ADRENAL MEDULLARY AND CORTICAL HORMONES IN MAN[1]

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Despite the anatomical contiguity of the cortex and the medulla of the adrenal gland, the functional relationship between the two parts of the gland in man has been insufficiently defined. Animal experiments have implied that the two embryologically dissimilar tissues may be functionally closely related. The dog, for example, has been found after adrenalectomy to be less sensitive to the pressor effect of infused noradrenaline (Ramey, Goldstein, and Levine, 1951) or adrenaline (Cleghorn, Fowler, Greenwood, and Clarke, 1950) than the intact animal. The pressor response of human normotensive subjects to noradrenaline has been shown to be increased by pre-treatment with deoxycorticosterone (Raab, 1942), and to be decreased in patients with adrenal insufficiency (am Ende, 1939). These and other observations have suggested that a functional relationship between the two parts of the adrenal gland may exist in man. Observations on a patient with a phaeochromocytoma, in whom adrenal insufficiency supervened, have confirmed the suspicion of such relationship. Additional experimental data are presented which throw further light on its mechanism.

## *Case Report*

Miss A. P. (AL 6551), aged 63, had been admitted to the Fulham Hospital in November 1958 under the care of Dr. A. L. Wyman. She gave a history of tiredness, breathlessness, and cough, of six weeks' duration, and of swelling of the ankles for three days. She had experienced attacks of very heavy sweating since a hysterectomy for fibromyomata in 1943.

*On admission* to Fulham Hospital she was in congestive cardiac failure, had an enlarged heart, with no murmurs, and a blood-pressure of 200/130. There were râles and small pleural effusions at both bases. The haemoglobin concentration was 14·7 g. per 100 ml. The white-cell count was 6,200 per cu.mm. The erythrocyte sedimentation rate was 2 mm. in one hour. The blood urea was 57 mg. per 100 ml. The serum electrolyte concentrations were normal. Glycosuria was present, and an oral glucose tolerance test showed a curve of diabetic type. A phentolamine test (5 mg. given intravenously) produced a fall of 30 mm. Hg in systolic blood-pressure. Pressor amines in the urine gave a positive result for phaeochromocytoma on a cat bio-assay (Mr. D. A. Willoughby). The patient's congestive failure responded to treatment with digoxin and

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diuretics. The symptoms and signs of phaeochromocytoma disappeared spontaneously, the specific tests became negative, and she was discharged from hospital.

She remained well until the following May (1959), when she began to perspire freely again, and her blood-pressure was found to have risen a little. The urine, assayed at University College Hospital, again showed the presence of catecholamines, and she was transferred to that hospital on 14. 7. 59 at the request of Dr. Wyman. Her complaints at this time were of excessive sweating by night and by day, summer and winter. No history of pallor or of palpitations could be elicited. She did not complain of headaches. There was no family history of hypertension.

*On examination* she was not in congestive heart failure. The heart was enlarged, with a presystolic gallop rhythm. Her blood pressure was 170/110. The fundi showed grade 2 hypertensive retinopathy. Râles were heard at the right base. The liver was enlarged to 2 cm. below the right costal margin. No abnormalities were noted in the nervous system. Laboratory investigation on admission showed haemoglobin 14·5 g. per 100 ml. The erythrocyte sedimentation rate was 32 mm. in one hour. The plasma concentration of sodium was 144 m-equiv., of potassium 4·1 m-equiv., of chloride 103 m-equiv., and of bicarbonate 25 m-moles per litre. The blood urea was 40 mg. per 100 ml., with a urea clearance of 85 per cent. of normal. The urine contained no albumin or sugar. Chest X-rays suggested left ventricular enlargement. Cat bio-assay for pressor amines in three separate specimens (Mr. D. A. Willoughby) strongly suggested the presence of a phaeochromocytoma (about 500 $\mu$g. noradrenaline equivalents per litre). Intravenous pyelography showed that the right kidney was rotated, but the cortical thickness and calycine pattern were considered to be normal. Presacral oxygen insufflation and tomography revealed a soft-tissue shadow above the left kidney suggestive of a tumour.

*Operation.* Before operation the patient was given phenoxybenzamine, 50 mg. a day by mouth for four days, to produce adrenergic blockade and thereby prevent a sudden increase of blood-pressure during operation. Exploration of the left adrenal failed to discover a tumour. The left adrenal was mobilized because a mass was felt in its substance, but this mass proved to be a kink in the adrenal artery. On the anterior surface of the left kidney an irregular tumour 2 cm. in diameter was found, and the kidney resected. This mass proved to be a localized area of fibrosis, atrophy, and cyst formation, probably formed by a small infarct. The operation site was closed without a phaeochromocytoma being found.

*After operation* the patient volunteered the information that she no longer sweated. Assays of her urine for pressor amines, however, showed that the excretion of these substances was as high as before. On the 14th day after operation she complained of dizziness on standing, and her standing blood-pressure was 95/65. On the following day she continued to be hypotensive, and fainted in the toilet. Her standing blood-pressure was 90/60; when recumbent it was 120/80. In spite of this the amount of pressor amines excreted in the urine continued to be high (500 $\mu$g. per litre). The electrocardiogram was unchanged. The plasma sodium concentration had fallen from 144 m-equiv. to 131 m-equiv. per litre, and the plasma potassium concentration had risen from 4·1 m-equiv. to 4·8 m-equiv. per litre. The patient became weak and lost appetite. Adrenal insufficiency was suspected, and a therapeutic trial of cortisone (50 mg. per day) was instituted. Immediately her appetite improved, and after two days her blood-pressure rose so that she could stand without feeling faint. Cortisone therapy was then stopped, and a corticotrophin test performed. There was very

little increase in the excretion of either 17-ketogenic steroids or 17-ketosteroids above control levels, which were within the lower part of the normal range; the response resembled that of partial adrenal insufficiency (Table I). Continued treatment with cortisone (37·5 mg. per day) resulted in an increase of blood-pressure to 160/100 in the recumbent and 100/70 in the standing position. The patient was discharged from hospital, taking 37·5 mg. of cortisone daily and

TABLE I

*Response to Stimulation by Corticotrophin in Patient A. P.*

(40 units of ACTH-gel given intramuscularly twice daily)

| *Day* | *17-ketosteroids (mg./24 hrs.)* | *17-ketogenic steroids (mg./24 hrs.)* |
|---|---|---|
| 1 (Control 1) . . | 5·6 | 6·8 |
| 2 ( „ 2) . . | 3·3 | 5·6 |
| 3 (ACTH 1) . . | 6·5 | 6·9 |
| 4 ( „ 2) . . | 8·1 | 3·3 |

0·25 mg. of digoxin twice daily. It was planned to explore the right adrenal during a subsequent admission. Two months later she became breathless on the slightest exertion, and then developed congestive heart failure. She was readmitted to hospital on 15.1.60.

*On admission* she was febrile, and sweating profusely. The blood-pressure was 160/95, with pulsus alternans. The heart was enlarged, and a triple rhythm was present. The electrocardiogram showed depression of the ST segments in chest leads. On succeeding days the patient became more and more drowsy and apathetic, and developed incontinence. She then complained of severe pain in the left leg, which on examination was cold, blue, and pulseless. As she was unfit for embolectomy she was given anticoagulation therapy. Shortly after this her condition improved remarkably, and a mid-thigh amputation could be carried out; but again her condition deteriorated, and she died on 8.2.60.

*Autopsy* revealed extensive myocardial scarring with mural thrombi, the source of her arterial emboli. The remaining kidney showed numerous infarcts. Macroscopically the right adrenal gland was entirely replaced by a phaeochromocytoma measuring $3·7 \times 2 \times 2$ cm. and weighing 10 g. The left adrenal gland was fibrotic and atrophic.

*Comment.* The conclusion drawn from observations on this patient was that the normal pressor activity of the catecholamines secreted by the phaeochromocytoma was ineffective in the face of a greatly reduced secretion of adrenocortical hormones. It was not possible to demonstrate whether the hypotension in the presence of an active phaeochromocytoma was due to the absence of hormones of glucocorticoid or of mineralocorticoid type, or both. Alternatively, a secondary alteration of metabolism, for example, sodium depletion, due to decreased secretion of adrenocorticoid hormones, may have altered the pressor response to catecholamines.

In order to explain the above observations, and to elucidate more precisely the mechanism of the pressor activity of catecholamines and the relationship to adrenocortical hormones, the results of further studies carried out on normal subjects and on patients with adrenal insufficiency are presented.

*Supplementary Studies*

1. *Pressor activity of noradrenaline in adrenal insufficiency.* Patient E. W., aged 49 years, had undergone total bilateral adrenalectomy for malignant hypertension. His blood-pressure fell after operation, but not to normal levels, and he remained well on a maintenance dose of 50 mg. of cortisone acetate daily. Some months later he was admitted to hospital with an adrenal crisis,

TABLE II

*Pressor Effect of an Infusion of Noradrenaline in a Patient with Adrenal Insufficiency*

| Dose (μg./kg./min.) | Increment of blood-pressure over base-line (mm. Hg) | |
|---|---|---|
| | No treatment | During hydrocortisone infusion |
| 0·1 | +10/8 | +12/6 |
| 0·2 | +30/7 | +22/6 |

TABLE III

*Pressor Effect of an Infusion of Noradrenaline in a Patient with Adrenal Insufficiency Before and After Treatment for 15 Days with Cortisone Acetate and Sodium Chloride*

| Dose (μg./kg./min.) | Increment of blood-pressure over base-line (mm. Hg) | |
|---|---|---|
| | Before treatment | After treatment |
| 0·1 | 8/8 | 32/28 |
| 0·2 | 43/25 | 44/30 |
| 0·3 | 62/35 | 75/37 |

dehydrated and weak; the plasma sodium was 132 m-equiv. per litre, plasma potassium 6·7 m-equiv. per litre, and blood urea 222 mg. per 100 ml. The blood-pressure was 148/100. Before any treatment was instituted he was given intravenous infusions of noradrenaline for 10-minute periods at rates of 0·1 and 0·2 μg. per kg. per minute. Hydrocortisone as the free alcohol was then infused in normal saline at a rate of 100 mg. per hour, and the noradrenaline infusions were repeated. The increment of blood-pressure during the latter two infusions compared with the control values (Table II) showed no significant difference. Continued treatment with hydrocortisone and saline ultimately restored the plasma electrolytes and blood urea to normal.

Patient J. N., aged 59 years, suffered from Addison's disease caused by adrenal tuberculosis. He was admitted to hospital for diagnosis, with a history of tiredness, weakness, and increasing pigmentation for nine months. The plasma sodium was 140 m-equiv. per litre, plasma potassium 4·95 m-equiv. per litre, blood urea 24 mg. per 100 ml., and resting blood-pressure 102/62. Before any treatment was instituted he was given intravenous infusions of noradrena-

line for 10-minute periods in doses of 0·1, 0·2, and 0·3 μg. per kg. per minute. The infusions were repeated after he had received 50 mg. of cortisone and 6 g. of sodium chloride daily for 15 days. His resting blood-pressure at this time was 101/54. The results are shown in Table III. The blood-pressure response to the infusion of noradrenaline at the smallest dosage (0·1 μg. per kg. per minute)

TABLE IV

*Pressor Effect of an Infusion of Noradrenaline in a Patient with Hypopituitarism compared with that in Normal Subjects*

| *Dose* (*μg./kg./min.*) | *Increment of blood-pressure over base-line* (*mm. Hg*) | |
|---|---|---|
| | *Hypopituitarism* | *Normal* |
| 0·05 | 44/37 | 8/10 |
| 0·10 | 48/32 | 22/19 |
| 0·15 | 79/75 | 31/26 |

was greatly increased by treatment. With the other dosages the responses were not greatly changed, although the blood-pressure readings were slightly higher after treatment.

The inference drawn from these two experiments was that the simultaneous infusion of hydrocortisone with noradrenaline did not increase the pressor activity of noradrenaline infused alone, but pre-treatment of the patient with cortisone and salt for 15 days did augment the response.

2. *Administration of noradrenaline to a patient with hypopituitarism.* Patient H. B., aged 36 years, was suffering from hypopituitarism following a severe post-partum haemorrhage nine years previously. The plasma sodium was 136 m-equiv. per litre, plasma potassium 4·75 m-equiv. per litre, and resting blood-pressure 109/65. Before treatment was instituted, noradrenaline was infused intravenously for 10-minute periods at rates of 0·05, 0·1, and 0·15 μg. per kg. per minute. The results are shown in Table IV. The mean blood-pressure response encountered in 10 normal subjects is shown in the third column. The patient's response to noradrenaline was greater than normal. The virtual absence of secretion of cortisol which occurs in hypopituitarism did not prevent the usual pressor activity of noradrenaline.

The difference in the blood-pressure response between the patients lacking adrenal secretion of both hydrocortisone and aldosterone and the patient with hypopituitarism (in whom the secretion of hydrocortisone would be reduced to about 10 per cent. of normal, but aldosterone secretion would be expected to be about 60 per cent. of normal (Ross, van 't Hoff, Crabbé, and Thorn, 1960) may be due to (*a*) the presence of aldosterone, or (*b*) a secondary effect of aldosterone, by alteration of the distribution of sodium. To differentiate between these two alternatives, the response to the pressor activity of noradrenaline was determined in a normal subject before and during a period of sodium deprivation.

3. *Effect of sodium deprivation on the response to noradrenaline.* A normal volunteer (E. J. R.) was given a rice diet for 14 days; the sodium intake during this period was 3 m-equiv. a day. During this period he had a negative sodium balance of 158 m-equiv., and lost 3·1 kg. in weight, equivalent to a contraction of the total body water of 3·1 litres. The amount of aldosterone excreted in the urine rose from a mean of 6 μg. in 24 hours during the control period, on a

TABLE V

*Pressor Effect of an Infusion of Noradrenaline in a Normal Subject when on a Normal Diet and when on a Rice Diet*

| Dose (μg./kg./min.) | Increment of blood-pressure over base-line (mm. Hg) | |
|---|---|---|
| | On normal diet | On rice diet |
| 0·05 | 8/5 | 2/5 |
| 0·10 | 15/11 | 12/10 |
| 0·15 | 36/16 | 22/17 |
| 0·20 | 40/18 | 28/20 |

normal sodium intake, to 24 μg. in 24 hours on the 14th day of the diet; this probably corresponded to a fourfold increase in the rate of aldosterone secretion. During the period of sodium deprivation the plasma sodium concentration fell from 141 m-equiv. to 136 m-equiv. per litre. Infusions of noradrenaline, at rates of 0·05, 0·10, 0·15, and 0·20 μg. per kg. per minute, were given for 10-minute periods during the normal intake of sodium and on the 14th day of the rice diet. The results are shown in Table V. The resting blood-pressure was 108/69 during the normal diet and 106/69 during the rice diet. Although little change was noted in the increment of diastolic blood-pressure, the increment of systolic pressure was less on the low-sodium diet. The presence of increased amounts of circulating aldosterone did not prevent a decrease of the pressor activity of noradrenaline on the low-sodium diet.

4. *Restoration of circulating volume in a sodium-deprived subject.* (*a*) A normal volunteer (W. W. W.) was given an aldosterone antagonist (spironolactone, 'aldactone') in a dosage of 1,000 mg. daily. After five days of therapy he was given a restricted sodium intake of 18 m-equiv. a day. On the 14th day of the restricted sodium intake, when he felt nauseated, lacking in appetite, and weak, his plasma sodium concentration had fallen from 140 m-equiv. to 125 m-equiv. per litre. On this day he was given intravenous infusions of noradrenaline for 10-minute periods, at rates of 0·1, 0·2, and 0·3 μg. per kg. per minute. He was then given 700 ml. of salt-free dextran intravenously, and the noradrenaline infusions were repeated, after which he was given one litre of 0·9 per cent. sodium chloride intravenously (154 m-equiv. of sodium), and the noradrenaline infusions were again repeated. A control infusion of the same doses of noradrenaline had previously been given when the subject was receiving a normal diet. The blood-pressure alterations are shown in Table VI. Treatment with a restricted sodium intake and spironolactone had reduced considerably the

response to noradrenaline. The infusion of 700 ml. of dextran, an amount calculated to restore the plasma volume to normal, had little effect on the response to noradrenaline. The infusion of a litre of normal saline returned the blood-pressure response towards, but not quite to, that seen during an infusion in the control period.

(*b*) Another normal subject (E. J. R.) was subjected to a Kempner rice diet

TABLE VI

*Pressor Effect of an Infusion of Noradrenaline in a Normal Subject on a Normal Diet and on a Low-Sodium Diet, after an Infusion of Dextran (700 ml.) and after Normal Saline (1 litre)*

| | *Increment of blood-pressure over base-line (mm. Hg)* | | | |
|---|---|---|---|---|
| | | *Low-sodium diet* | | |
| *Dose (μg./kg./min.)* | *Normal diet* | *Diet only* | *+Dextran* | *+NaCl* |
| 0·1 | 12/12 | 2/4 | — | — |
| 0·2 | 20/18 | 14/12 | 12/12 | 18/24 |
| 0·3 | 42/26 | 18/18 | 18/20 | 28/24 |
| Haematocrit level % | | 51 | 46 | 41·5 |

TABLE VII

*Pressor Effect of an Infusion of Noradrenaline in a Normal Subject before and when taking a Rice Diet, and after an Infusion of Dextran when on a Rice Diet*

| | *Increment of blood-pressure over base-line (mm. Hg)* | | |
|---|---|---|---|
| *Dose (μg./kg./min.)* | *Normal diet* | *Rice diet* | *+Dextran* |
| 0·1 | 15/11 | 8/8 | 8/6 |
| 0·2 | 36/16 | 12/10 | 14/8 |
| 0·3 | 41/18 | 26/12 | 28/8 |

TABLE VIII

*Pressor Effect and Change of Plasma Sodium Concentration in Response to the Infusion of Noradrenaline (0·2 μg./kg./min.) in Four Normotensive Subjects*

| *Subject* | *Initial blood-pressure* | *Increment of blood-pressure (mm. Hg)* | *Fall of plasma sodium concentration (m-equiv./l.)* |
|---|---|---|---|
| 1 | 122/70 | 36/40 | 4·9 |
| 2 | 127/62 | 17/20 | 1·3 |
| 3 | 108/70 | 50/26 | 2·3 |
| 4 | 112/62 | 48/37 | 3·7 |

for five days (sodium intake 3 m-equiv. a day) together with spironolactone, 1,000 mg. a day. Noradrenaline infusions were given for 10-minute periods at rates of 0·1, 0·2, and 0·3 μg. per kg. per minute before starting the diet and on the fifth day. An infusion of 540 ml. of sodium-free dextran was then given to restore the plasma volume. As in the other normal subject, this infusion had virtually no effect on the pressor activity of infused noradrenaline (Table VII).

5. *Changes of plasma sodium and potassium concentration during an infusion of noradrenaline.* Noradrenaline, in a dosage of 0·2 μg. per kg. per minute, was infused into four normotensive volunteers for a period of 30 minutes. Venous blood was taken for estimation of sodium and potassium concentrations at intervals of approximately five minutes before, during, and after the infusion. Changes in blood-pressure and in plasma sodium and potassium concentration in one of these experiments are shown in Fig. 1. Changes in electrolyte concentration are shown in Table VIII; these figures are the means of readings obtained before and during the noradrenaline infusions. The mean increases in systolic and diastolic blood-pressure have been plotted against the fall of plasma sodium concentration in Fig. 2. The results show that the rise in diastolic pressure is related to the extent of the fall of plasma sodium concentration.

## *Discussion*

The experimental observations outlined above clearly indicate that the modification of the pressor activity of noradrenaline by adrenal steroids in man is not due to potentiation by their simultaneous presence, but to secondary alterations caused by the prolonged presence or absence of the steroids. These observations are not in agreement with those of Ramey, Goldstein, and Levine (1951) and Fritz and Levine (1951), who found that cortical hormones given simultaneously with catecholamines caused potentiation of the pressor effect in animals, nor with those of Rukes, Orr, Forsham, and Galante (1955) in similar circumstances in man. Other observers have agreed that prolonged pre-treatment with adrenal corticoid or corticotrophin potentiates the pressor activity of catecholamines, whereas in the acute experiment no potentiation is observed (Dustan, Corcoran, Taylor, and Page, 1950; Kurland and Freedberg, 1951).

Ramey and Goldstein (1957) have reviewed the functional relationship of adrenocortical steroids and catecholamines. They concluded that adrenocortical steroids are 'permissive' for the action of catecholamines, and that in the absence of steroids the threshold of response to catecholamines is raised at the end-organ. The validity of such a conclusion depends upon the definition of the word 'permissive', and the use of such a term does not explain the mechanism whereby the two secretions of the adrenal gland act synergically. It is agreed that the pressor activity of catecholamines is decreased by adrenalectomy (Coombs, 1925, and many subsequent authors), and it has been shown that the pressor effect of pre-treatment with deoxycorticosterone acetate is enhanced by simultaneous administration of an increased sodium intake (Raab, 1942) and decreased by simultaneous sodium deprivation (Raab, Humphreys, Makous, DeGrandpré, and Gigee, 1952). Pre-treatment with deoxycorticosterone must, however, be of some days' duration to be effective. The simultaneous administration of deoxycorticosterone and catecholamine does not result in potentiation of pressor effect unless the subject is pre-treated with deoxycorticosterone (Schürger, 1947). Furthermore, the administration of deoxycorticosterone acetate during the period of sodium deprivation failed to reverse the

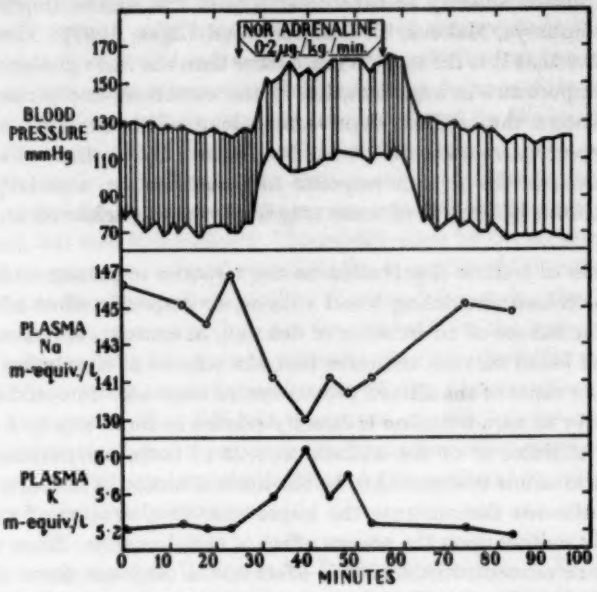


FIG. 1. Changes in blood-pressure and plasma potassium concentration during the infusion of noradrenaline in a normal subject.

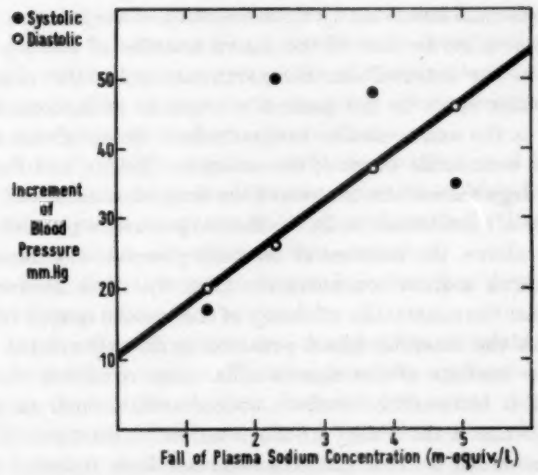


FIG. 2. Relationship between the rise of systolic and diastolic blood-pressure and the fall of plasma sodium concentration during noradrenaline infusions (0·2 μg. per kg. per minute) in a normal subject.

decreased pressor activity of catecholamines in the sodium-deprived patient (Raab, Humphreys, Makous, DeGrandpré, and Gigee, 1952). These observations indicate that it is the sodium ion, rather than the mere presence of steroid, that is of importance in augmentation of the catecholamine pressor response. It is well known that sodium deprivation alone will lower the blood-pressure in many hypertensive patients. It has been shown above that sodium deprivation will decrease the pressor response to noradrenaline, especially when the negative sodium balance is of some magnitude, as was achieved in the subject W. W. W.

The effects of sodium deprivation on the response to pressor amines may be due to a decreased circulating blood volume, or a specific effect of the lack of sodium. The failure of an infusion of dextran, in amounts sufficient to restore the depleted blood volume, indicates that the volume of circulating fluid is not primarily the cause of the altered pressor-amine response. It would appear that the sensitivity to noradrenaline is directly related in some way to a decrease of total body sodium, or of the sodium content of some compartment of body water. The arteriole is assumed to be the locus of action of noradrenaline. The above experiments demonstrate the importance of alteration of some moiety of total body sodium upon the pressor effect of noradrenaline. Since variation of plasma sodium concentration had little effect on the response, these observations imply that the mechanism of the pressor activity of this catecholamine might be dependent upon the intracellular concentration of sodium within the arteriolar wall.

The infusion of noradrenaline in man results in a lowering of the plasma sodium concentration, as shown in Fig. 1. Since the plasma sodium concentration returns to normal immediately upon cessation of the infusion, the alteration of concentration must be due to the active transfer of sodium ions from the extracellular to the intracellular compartment under the influence of noradrenaline, rather than to the passive movement of sodium ions from the intravascular to the extravascular compartment. Some of this sodium enters the cells of the contractile tissue of the arteriole. Tobian and Fox (1956) have shown that in dogs the sodium content of the femoral arterial wall was increased by the infusion of noradrenaline. In the four experiments on normotensive subjects recorded above, the increase of diastolic pressure was directly related to the fall in plasma sodium concentration (Fig. 2). It is inferred from these experiments that the contractile efficiency of the smooth muscle of the arteriolar wall, and hence the diastolic blood pressure, is directly related to the intracellular sodium content of the muscle cells. Any condition that leads to a reduction of this intracellular sodium concentration, such as adrenocortical insufficiency, weakens the ability of the arteriole to contract. Conversely, an increased intracellular sodium concentration has been reported in association with hypertensive states. Thus an increased sodium content has been found in the wall of the renal artery of hypertensive patients studied after death (Tobian and Binion, 1952), and in the wall of the aorta in rats with experimental renal and deoxycorticosterone-induced hypertension (Tobian and Binion, 1954;

Tobian, 1956). The excessive response to the administration of noradrenaline shown by the patient with hypopituitarism was unexpected; if the above hypothesis be true, it would indicate that in hypopituitarism the intracellular sodium concentration is increased. Evidence from balance data and muscle biopsies in cases of hypopituitarism has been produced by Waterhouse, Keutmann, and Fenniger (1952) to show that this is the case.

The present series of studies on the human subject indicates that there is a functional relationship between the hormones secreted by the two parts of the adrenal gland, but that it is indirect. The modification by the cortical hormones of the pressor response to infused noradrenaline is mediated through their effect on the concentration of sodium within the arteriolar wall; the greater the intracellular sodium concentration, the greater will be the pressor response. Absence of adrenocortical hormones ultimately leads to depletion of the intracellular sodium content, and to weakening of the pressor effect of adrenal medullary hormones. The different responses shown by the patient with Addison's disease and by the patient with hypopituitarism suggest that aldosterone may be of more importance than hydrocortisone in regulating the intracellular sodium concentration.

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*Summary*

Observations are reported on a patient with persistent hypertension due to a phaeochromocytoma, in whom adrenal insufficiency developed. Severe hypotension resulted, which was corrected by the administration of cortisone.

Supplementary studies, to explain the inability of noradrenaline secreted by the tumour to exert a pressor effect, showed that:

1. In acute adrenal insufficiency the simultaneous infusion of hydrocortisone with noradrenaline had no greater pressor effect than noradrenaline alone. Replacement therapy with cortisone and sodium chloride for 15 days increased the pressor response to noradrenaline in this patient.

2. A greater-than-normal response to noradrenaline was shown by a patient with untreated hypopituitarism.

3. Dietary deprivation of sodium in normotensive subjects weakened the pressor response to noradrenaline. This response was unaffected by restoration of plasma volume by salt-free dextran, but was restored to normal by the infusion of sodium chloride. Sodium depletion by means of an aldosterone antagonist and a low-sodium diet also weakened the pressor response to noradrenaline.

4. The infusion of noradrenaline decreased plasma sodium concentration, and increased plasma potassium concentration. The rise of diastolic pressure during the noradrenaline infusion was directly related to the fall of plasma sodium concentration.

The conclusions drawn are that the modification of the pressor activity of noradrenaline by adrenal cortical hormones is not due to potentiation by their simultaneous presence, but to modification of intracellular sodium concentration in the smooth muscle of the arteriolar wall. The greater the cell sodium content, the greater is the pressor response.

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# A STUDY OF THE EFFECTS OF ALBUMIN INFUSIONS IN PATIENTS WITH CIRRHOSIS OF THE LIVER[1]

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Ever since the close relationship between the lowered concentration of serum albumin and the prognosis in chronic liver disease has been recognized (Post and Patek, 1942*a*) it has been accepted that one of the chief aims of therapy is to increase the intake of nitrogen and attempt to return the serum albumin level to normal. When this can be accomplished by dietary means the prognosis has been shown to be considerably improved (Patek, Post, Ratnoff, Mankin, and Hillman, 1948). In a large number of patients, however, the serum albumin does not increase even though a markedly positive nitrogen balance has been achieved (Post and Patek, 1942*b*), and in some it is impossible to raise the dietary protein level above a sub-optimal level, as higher intakes lead to attacks of portal-systemic encephalopathy. In such cases the serum albumin remains diminished, or falls further to levels at which ascites and oedema usually occur (Ricketts, 1951).

Attempts to raise the concentration of serum albumin by intravenous infusions were first made by using plasma or the patient's own ascitic fluid (Greene, 1943; Ricketts, 1951), a limited success being achieved and diuresis occurring in some cases. The practical difficulties of the latter method have not encouraged its general use, and in any case it only eliminates the loss of protein from the body by paracentesis without raising the total body content. When albumin was successfully produced from human serum in sufficient quantities, and could be adequately sterilized without denaturation, it was reasonable to hope for a major advance in the management of chronic liver disease from its use. It was first administered intravenously to patients with cirrhosis of the liver by Janeway, Gibson, Woodruff, Heyl, Bailey, and Newhouser (1944), who showed some rise in serum albumin concentration and considerable improvement in well-being, but failed to produce a diuresis. Kunkel, Labby, Ahrens, Shank, and Hoagland (1948), and Post, Rose, and Shore (1951), in studies of larger groups of such patients, reported that ascites and oedema could be eliminated in more than 50 per cent. of their cases; but this rate of success was not universally obtained, and several small series (Thorn, Armstrong, and Davenport, 1946; Armstrong, 1948; Mankin and Lowell, 1948; Patek, Post, Ratnoff, Mankin, and Hillman, 1948; Faloon, Eckhardt, Murphy, Cooper, and Davidson,

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1949; Goodyer, Peterson, and Relman, 1948–9; Watson and Greenberg, 1949; Havens and Bluemle, 1950; Ricketts, 1951) present a variable pattern of response. Of the two large and relatively successful series, a complete diuresis was obtained in 11 of the 15 patients in the first series and 21 of 34 in the second. Of the patients who responded adequately, the majority developed ascites again as the serum concentration fell, and they were then given further courses, which again resulted in a complete diuresis. In one patient maintenance infusions were performed satisfactorily for a year. Other investigators reported similar results, but with less success, and in some instances the incidence of complications outweighed the benefits derived. Perhaps because of this, and in spite of the more favourable reports, the clinical use of albumin appears to have lapsed, and no further reports of its use have been published. It may also be that the difficulty and expense of its production and the availability of more satisfactory diuretics (Shaldon, McLaren, and Sherlock, 1960) have militated against its more general use.

There is, however, a group of patients with fluid retention due to chronic liver disease who eventually prove resistant to conventional therapy as a regular out-patient measure, and who continue to accumulate ascites or oedema, or both, in spite of strict dietary salt restriction and the use of diuretics. It seemed that the hopeless prognosis of this group compelled further trial of albumin infusions in an attempt to reverse the rapid clinical deterioration. It also seemed likely that close observation of the physiological and biochemical changes accompanying a period of repeated infusions, and particularly at the time of diuresis, might yield some information as to the pathogenesis of ascites, the mechanism of the diuresis, and the equilibrium relationships between the plasma and the ascitic fluid. In the present study salt-free human albumin was given intravenously to a series of 13 such patients. All had ascites or oedema, or both, and were greatly limited in their ability to excrete sodium in response to the load imposed by a normal diet. In the majority of patients given an adequate course of infusions ascites and oedema resolved fully, and in all these patients maintenance therapy was given by further infusions at intervals of one to two weeks for up to two and a half years, with continued control. Furthermore, there was profound general symptomatic improvement, which persisted throughout the period of maintenance. No spontaneous rise in serum albumin concentration was seen during this follow-up period, and it seems likely that the rate of synthesis did not recover with the general improvement in nutrition. The other complications of the disease also continued to occur and to carry their own risk; and their further incidence was largely unaffected by albumin infusions. The failure of some cases to respond to albumin therapy emphasized the absence of knowledge as to the mechanism of its action; it is felt that careful observations of protein concentrations, colloid osmotic pressure values in plasma and ascitic fluid, and transcapillary diffusion and metabolic turnover of albumin, throw some light on this and other problems. The full results of the last-named investigation will be reported at a later date.

### *Methods*

The albumin was provided by the Blood Products Laboratory, Lister Institute of Preventive Medicine. It was prepared from pooled plasma by an ether fractionation process, heat sterilized, and supplied in the dried state, each unit containing 25 g., to be made up for intravenous use to 100 ml. with 5 per cent. dextrose and given through standard Blood Transfusion Service sets. Initial infusions were made with 25 g. lots given over one to two hours at daily intervals until the preliminary course was complete; occasionally, when resistance to therapy was met, an increase to 100 g. per infusion was made. Maintenance infusions were of 50 g. or 75 g. each, and given to out-patients in approximately one hour.

Plasma volume was estimated by intravenous injection either of T-1824 or of $^{131}$I-labelled albumin (Mollison, 1956). Red-cell mass and total circulating blood volume were deduced from the plasma volume and the venous haematocrit level, the latter being corrected to true body haematocrit level. Assumptions about further changes in blood volume were made from changes in venous haematocrit value, provided there was no evidence of haemolysis or bleeding. On a number of occasions these changes were checked by further plasma-volume estimations, and satisfactory agreement was obtained. Haematocrit estimations were performed by centrifuging at 3,000 r.p.m. for 30 minutes (radius 9 cm.). The volume of the ascitic fluid was estimated by injection of T-1824 or $^{131}$I-labelled albumin into the peritoneal cavity and, after making some effort to obtain adequate mixing by manipulation of the abdomen, samples were withdrawn 20 and 30 minutes later from the opposite side of the abdomen. Total protein concentration was estimated by the biuret method, compared against a standard estimated by the micro-Kjeldahl technique, and the albumin and $\gamma$-globulin figures were obtained by electrophoretic separation of the different protein fractions on filter paper (Hardwicke, 1954). Colloid osmotic pressure was estimated in the Rowe osmometer (Rowe and Abrams, 1957). Measurements of body weight and abdominal girth (at the level of the umbilicus) were taken daily, and 24-hour collections of urine made and the sodium and potassium content estimated. These latter values were obtained, as were serum values, by the method of flame photometry. Albumin labelled with $^{131}$I for volume and metabolic studies was the same as that used for regular infusions, and iodinated by a modification of the method described by McFarlane (1956).

### *Patients Treated*

Thirteen patients were selected for therapy (Table I*a*), and had been known to be suffering from cirrhosis of the liver for intervals varying from three months to 10 years; the diagnosis was based on clinical and biochemical findings, and histological confirmation was obtained by biopsy or autopsy examination in 10 cases. Ascites or oedema, or both, were present in every case, and had been treated with a dietary course of high protein intake and salt restriction. It had

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*Summary of Clinical and Biochemical Findings (Table*

*Table Ia*

| *Patient* | *Age (years)* | *Sex* | *Length of clinical history (months)* | | *Ascites* | *Oedema* | *Aetiological history* | *History of portal-systemic encephalopathy* | *Evidence of portal hypertension* | *Serum albumin (g./100 ml.)* | *Serum γ-globulin (g./100 ml.)* | *Serum COP (cm. water)* | *Serum bilirubin* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Cirrhosis* | *Fluid retention* | | | | | | | | | |
| I | 61 | M | 18 | 2 | ++ | + | Alcohol | + | Varices, haematemesis | 2·6 | 2·5 | 21 | 1 |
| II | 63 | M | 48 | 48 | ++ | — | Nil | — | Varices | 2·5 | 3·4 | 26·5 | 0 |
| III | 65 | M | 12 | 4 | ++ | + | Alcohol | + | Varices, haematemesis | 2·0 | 2·4 | 25 | 1 |
| IV | 46 | M | 4 | 2 | ++ | + | Alcohol | + | Varices, splenomegaly | 2·3 | 2·8 | 23 | 1 |
| V | 60 | F | 120 | 18 | ++ | + | Hepatitis | + | Varices | 1·0 | 3·0 | 26 | 4 |
| VI | 33 | F | 4 | 4 | ++ | — | Nil | — | Splenomegaly | 2·0 | 4·6 | 24 | 6 |
| VII | 65 | F | 3 | 1 | ++ | + | Nil | — | Varices, splenomegaly | 2·6 | 1·5 | 21 | 6 |
| VIII | 42 | M | 12 | 2 | + | ++ | Alcohol | — | Varices, splenomegaly | 2·1 | 1·0 | .. | 3 |
| IX | 52 | M | 9 | 6 | + | ++ | Alcohol | + | Nil | 2·3 | 2·4 | 17 | 0 |
| X | 25 | F | 108 | 30 | — | + | Hepatitis | + | Varices | 1·6 | 3·7 | 18·5 | 2 |
| XI | 50 | M | 46 | 42 | ++ | — | Nil | — | Varices, haematemesis | 1·8 | 2·4 | 24 | 0 |
| XII | 53 | M | 36 | 3 days | ++ | + | Alcohol | + | Varices | 2·2 | 3·3 | 21 | 3 |
| XIII | 50 | M | 9 | 2 | ++ | ++ | Nil | — | Splenomegaly | .. | .. | 26·5 | 40 |

COP = colloi

TABLE I

*Table Ia), and of the Response to Albumin Infusions (Table Ib).*

| *Serum bilirubin (mg./100 ml.)* | *Prothrombin index (% of normal)* | *Serum sodium (m-equiv./l.)* | *Plasma volume (ml./kg. body weight)* | *Blood volume (l./m.² height)* | *Table Ib* — *Albumin infused (g.)* | | *Diuresis* | *Amount of albumin given (g.) before sodium balance became negative* | *COP of plasma at time of diuresis (cm. water)* | *Survival time (months)* | *Mode of death* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Primary course* | *Maintenance* | | | | | |
| 1·5 | 60 | 126 | 42 | 0·94 | 1,200 | 0 | Nil | No diuresis | No diuresis | 3 | Coma |
| 0·5 | 100 | 129 | 39 | 0·91 | 1,350 | 0 | Nil | ,, | ,, | 1 | Coma |
| 1·9 | 70 | 126 | 59 | 0·90 | 1,800 | 0 | Partial | ,, | ,, | 3 | Haemorrhage and coma |
| 1·5 | 70 | 130 | 51 | 1·34 | 1,650 | 2,400 | Complete | 350 | 26 | 12 | Syncope following fever |
| 4·0 | 50 | 126 | 49 | 1·17 | 1,250 | 4,000 | ,, | 600 | 41 | 30 | Coma |
| 6·0 | 40 | 131 | 63 | 1·12 | 700 | 4,750 | ,, | 75 | 25 | Alive 36 | — |
| 6·4 | 37 | 130 | 49 | 1·10 | 1,150 | 450 | ,, | 350 | 32 | 4 | Haemorrhage and coma |
| 3·7 | 54 | 140 | 59 | .. | 700 | 1,200 | ,, | 100 | ? | 12 | Coma |
| 0·7 | 55 | 130 | 63 | 1·51 | 500 | 1,750 | ,, | 25 | 20 | 9 | Syncope following fever |
| 2·1 | 40 | 141 | 61 | 1·26 | 900 | 3,300 | ,, | 50 | 20 | Alive 18 | — |
| 0·5 | 70 | 125 | 56 | 1·02 | 650 | 0 | Nil | No diuresis | No diuresis | 1 | Shock |
| 3·4 | 50 | 109 | 57 | 1·40 | 100 | 0 | Nil | ,, | ,, | 3 days | Coma |
| 40·0 | .. | 123 | .. | .. | 300 | 0 | Nil | ,, | ,, | 5 days | Coma |

colloid osmotic pressure.

proved possible to control or eliminate the retained fluid in some patients for considerable periods but, when referred for albumin therapy, all except one showed ascites or oedema of disabling proportions which it had proved impracticable to control other than by keeping the patient in hospital. Patient X had no ascites and only a little oedema, and had been kept moderately well controlled for 18 months with diuretic therapy. She was, however, failing in general well-being, and each intercurrent infection produced moderate oedema and severe malaise, forcing her to relinquish her work. All 13 patients presented a very advanced pattern of the disease, 10 showing evidence of portal hypertension with splenomegaly or oesophageal varices. A history of episodes of portal-systemic encephalopathy was elicited in seven patients, of whom two had been in hepatic coma and five were maintained free from these attacks only by continuous antibiotic therapy or dietary protein restriction. Biochemical assessment revealed serum albumin concentrations varying from 1·0 to 2·6 g. per 100 ml., and $\gamma$-globulin from 1·0 to 4·6 g. per 100 ml. No patient had a normal prothrombin index, and the serum bilirubin was normal in only two, the highest figure recorded being 40 mg. per 100 ml. (Patient XIII). The patients were given their initial course of infusions in the Queen Elizabeth Hospital, Birmingham. They were (except patients II, VIII, and X) maintained on low-salt diets (20 to 50 m-equiv. of sodium per day) containing as much protein as the patient would tolerate (up to 120 g. per day). Diuretics were not given (except in patient V), so that the onset and causes of the diuresis might be studied with as few complicating factors as possible.

### *Clinical Results*

1. *Effects on fluid retention.* The major clinical effect of albumin infusions has been the elimination of ascites and oedema in the majority of patients. After the diuresis these patients have all been discharged from hospital taking normal amounts of sodium, and while receiving maintenance infusions have remained free from fluid retention without diuretics. Table I*b* shows the response to therapy of all the patients studied; the first 10 either responded successfully or reached a serum albumin concentration of at least 4·8 g. per 100 ml. before failure was conceded. The last three patients died at some time during the course of therapy, and it is not therefore possible to say whether or not a diuresis would have occurred if an adequate trial had proved possible. In the 10 patients who received a full course of infusions oedema was always eliminated, whereas a complete diuresis occurred in only four of the seven patients who had considerable ascites.

The exact time-relationships between the commencement of infusions and the onset of diuresis are illustrated in Fig. 1, where the three selected criteria of success—sodium excretion, body weight, and abdominal girth—are charted for each patient. It is seen that urinary sodium presented the first sign of impending diuresis and, while it was generally accompanied by changes in weight, diminution in girth was often considerably delayed, and might even be

increasing at a time when the two other indices showed signs of improvement (patients IV and VII). The commencement of this natriuresis was always seen within the first few days when appreciable oedema was present. Once the urinary sodium had shown a significant increase, the complete elimination of the oedema or ascites was rapid and complete within 14 days. The diuresis seen in patient V is of note, as continuation of mercurial treatment throughout the course of infusions showed that the first sign of a diuresis was on the days of these mercurial injections, and so a diuretic responsiveness returned before a true spontaneous diuresis took place. The diuretic was eventually withdrawn, and the patient was discharged with a normal intake of sodium.

2. *Other beneficial effects.* Another and equally striking effect of albumin infusions was the greatly increased sense of well-being which these patients developed. In eight of the 10 patients who received a full course of treatment, symptoms of weakness, malaise, and anorexia, which had been severe and disabling, disappeared after the first few infusions; all these patients felt stronger and more cheerful than they had for a considerable period (in some instances from the onset of the illness). The gain in well-being was much greater than could be accounted for by the hope generated by a new form of therapy, and indeed was independent of its success or failure, occurring as commonly in those who had a successful diuresis as in those who did not. This improvement has persisted in the patients who have since survived, and has been accompanied by a considerable increase in appetite and rapid repair of body wasting. Another disability was overcome in patient VIII, who had been impotent for three years; he regained his sexual potency within a few weeks of commencing infusions, and retained it for the remainder of his life. Seven of the 13 patients had episodes of portal-systemic encephalopathy, and on this account were being subjected to limitation of dietary protein or had been given antibiotics. Albumin infusions produced no aggravation of the neurological status in any patient, in spite of the intravenous administration in some instances of more than the maximal daily amount tolerated orally. The quantity of protein in the diet was maintained during therapy at its previous level; the total intake was therefore raised in some cases from 40 g. to 140 g. per day, whereas an increase of as little as 20 g. in the daily oral intake alone had in some instances proved sufficient to induce neurological deterioration. In two instances (patients V and X) attacks of portal-systemic encephalopathy were seen at and just before the commencement of albumin infusions; these attacks disappeared completely, and have not returned, in spite of an increase in the amount of protein permitted in the diet.

In addition to the beneficial clinical effects, there has occasionally been some biochemical evidence of improvement in liver-cell function. Four patients (V, VI, VII, and VIII) with raised serum-bilirubin levels have been studied over a prolonged period during maintenance treatment with albumin, and a decrease has been seen in only one case—that of patient V, in whom the serum concentration remained at its original level of 4·5 mg. per 100 ml. for 12 months, and

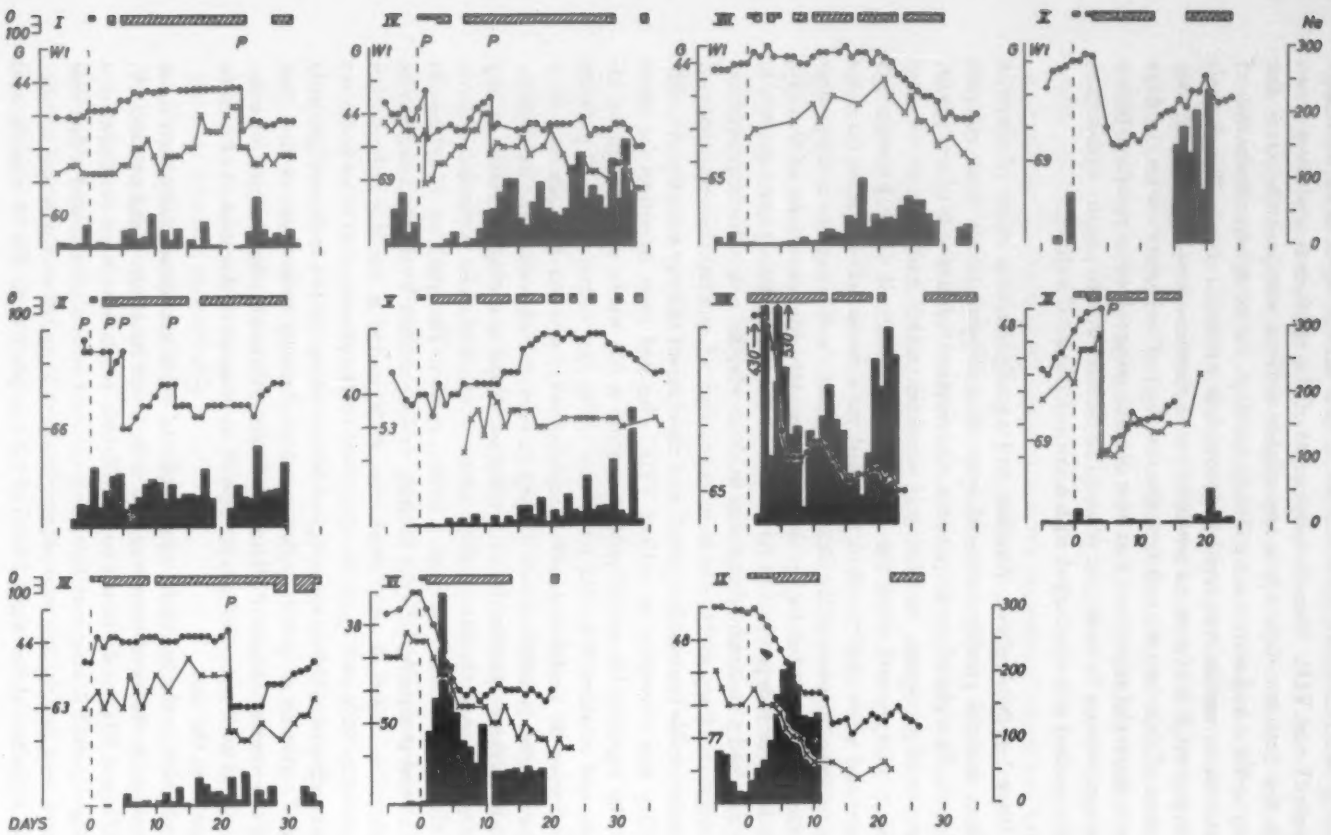


FIG. 1. Changes in body weight (●———●), girth (x———x), and 24-hour urinary sodium excretion (solid blocks) in patients I to XI during the period of daily albumin infusions (hatched blocks). The left-hand ordinate shows weight (Wt., one division = 3 kg.) and girth (G., one division = 2 inches). The right-hand ordinate shows sodium excretion (Na, m-equiv. per day); the absence of the zero abscissa line signifies that no estimation was made on that day. The rate of albumin infusion is indicated in g. per day. Paracentesis of the abdomen is indicated by P.

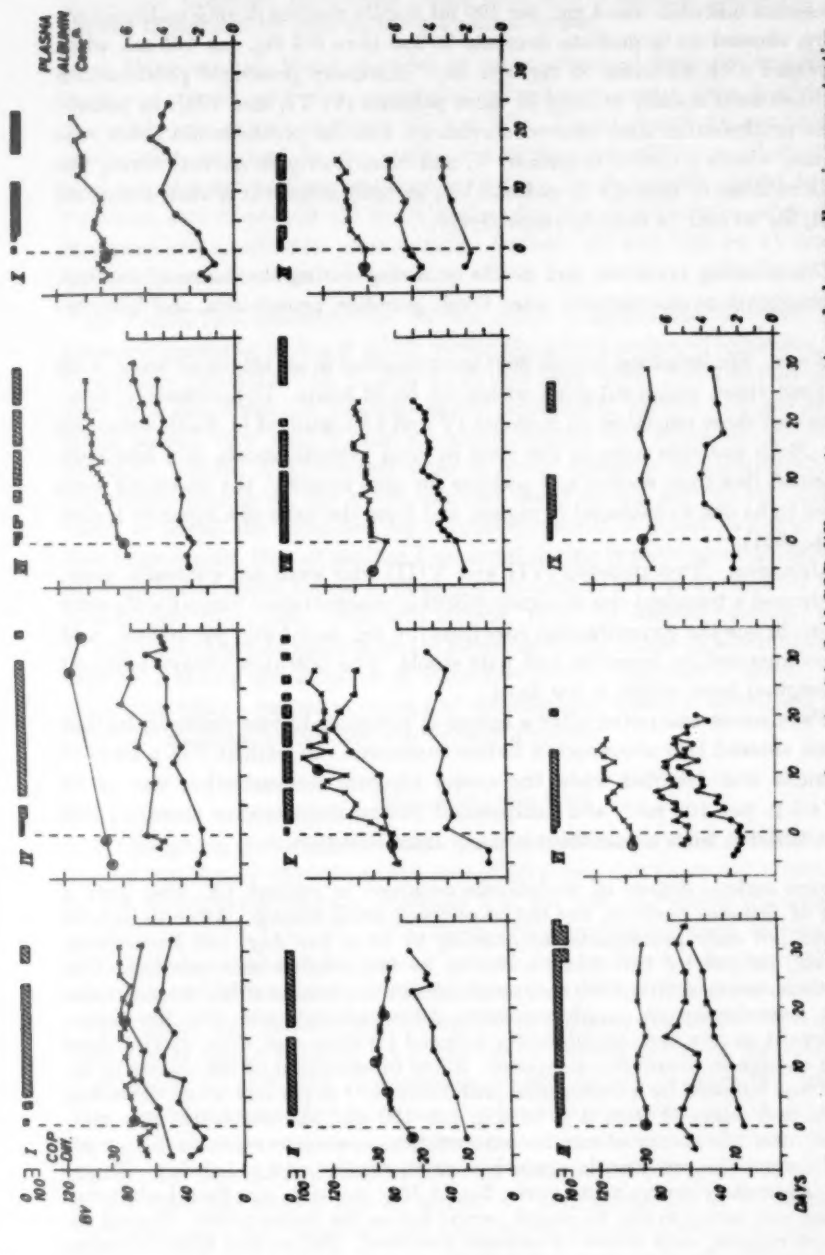


FIG. 2. Changes in blood volume (×——×), plasma/ascitic-fluid COP difference (▲——▲), and plasma albumin concentration (●——●) in patients I to XI during the period of daily albumin infusions. The left-hand ordinate shows blood volume (BV, ml./kg.) and COP difference (COP Diff., cm. water). The right-hand ordinate shows plasma albumin concentration (g./100 ml.). The rate of albumin infusions (g./day) is indicated by hatched blocks. Blood-volume estimations based on plasma-volume determinations performed that day are identified separately (⊛).

then dropped to below 2 mg. per 100 ml., where it then remained. Patient VI, whose serum bilirubin was 4 mg. per 100 ml. for six months during maintenance therapy, showed an immediate decrease to less than 0·5 mg. per 100 ml. when also treated with cortisone 50 mg. per day. Markedly prolonged prothrombin times have been serially studied in three patients (V, VI, and VII): in patient VII the prothrombin time showed no change, but the prothrombin index rose to normal within a month in patient V, and more slowly to normal during the first six months of therapy in patient VI; in both instances it then remained normal, for 30 and 24 months respectively.

3. *Complicating incidents and deaths occurring during the course of therapy.* The complications encountered were fever, jaundice, proteinuria, and haemorrhage.

(1) *Fever.* Six infusions (out of 500) have resulted in an attack of fever, with or without rigor, which subsided within six to 24 hours. Unfortunately, however, two of these reactions (in patients IV and IX) resulted in death within 36 hours. Both patients were at the time in good general health, and had been maintained free from ascites and oedema for nine months; the reactions were believed to be due to bacterial pyrogens, and form the basis of a separate report (Dykes, 1961).

(2) *Jaundice.* Two patients (VII and VIII) who were not clinically jaundiced showed a transient rise in serum-bilirubin concentration immediately after infusion; in one the concentration rose from 1·5 mg. to 3·4 mg. per 100 ml., and was accompanied by jaundice and pale stools. The bilirubin always returned to its original level within a few days.

(3) *Proteinuria* was noted after a course of infusions in two patients, neither of whom showed this abnormality before treatment. In patient VII a trace of proteinuria was detected when the serum albumin concentration was at its peak (4·5 g. per 100 ml.), and disappeared during maintenance therapy; this patient suffered from a persistent urinary tract infection.

A more serious degree of proteinuria occurred in patient IX, who gave a history of diabetes mellitus, but not of previous renal disease. After 10 days of infusions not only proteinuria, amounting to 10 g. per day, but haematuria appeared, judged by the Addis count to be 950,000,000 cells per day. The proteinuria was selective, with no loss of the serum proteins of higher molecular weight (caeruloplasmin, $\alpha_2$-glycoprotein, $\beta$-lipoprotein), nor was the serum complement at any time significantly reduced (Walton and Ellis, 1958); there was no change in creatinine clearance. Since interruption of the course of infusions was followed by a decrease in proteinuria to 1 g. per day when the serum albumin had relapsed from 4 g. to 3 g. per 100 ml., the treatment was reinstituted, and the serum albumin concentration rose once more to 3·4 g. per 100 ml., when the proteinuria again increased, reaching 50 g. per day. Thereafter a moderately severe proteinuria, 2 g. to 15 g. per day, was found whenever the urine was tested in the 10-month period before the patient died. Except for one short relapse, only traces of oedema persisted. Before and after infusions a mild hypertension (160/100 to 140/90) was recorded, without evidence of exacerbation at the time of heavy proteinuria. At autopsy the renal veins

appeared normal, but histologically there was very slight thickening of the glomerular basement membrane; there were also occasional clusters of mononuclear inflammatory cells in the interstitial tissue.

(4) *Haemorrhage.* Major episodes of gastrointestinal haemorrhage occurred in three patients, and were considered to be due to peptic ulcer (patient VII) or oesophageal varices (patients III and XI). The haemorrhage resulting from peptic ulcer occurred in the period of maintenance infusions. Haemorrhage from oesophageal varices happened in patient XI during the initial course of infusions, and in patient III three weeks after the initial course, when he was receiving 100 g. albumin once a week. Patient XI had bled on 11 occasions before albumin treatment.

(5) *Deaths* not accounted for by the above complications occurred in two instances (patients XII and XIII) during the initial course of infusions, and in four others (patients I, II, V, and VIII) at a later date. The first two patients were both desperately ill when infusions commenced: patient XII was accumulating ascites very rapidly, and his serum sodium had fallen to 109 m-equiv. per litre; patient XIII showed signs of portal-systemic encephalopathy, and was deeply jaundiced, the serum bilirubin being 40 mg. per 100 ml. Infusions failed to arrest the deteriorating clinical condition in either patient. Of the four later deaths, that of patient I occurred during hepatic coma three months after an unsuccessful course of infusions; patient II died in coma after an ileoentectrophy (Neumann, Adie, and Hinton, 1957) for resistant ascites; patient V, after two years of excellent health following the initiation of albumin therapy, suffered a severe exacerbation of ulcerative colitis, and died in coma; patient VIII, also after a period of successful maintenance therapy, developed portal-systemic encephalopathy and died within a month.

### *Biochemical Findings*

1. *Changes in protein concentration in serum and ascitic fluid.* After the intravenous infusion of albumin at the rate of 50 g. per day there was a steady rise in the serum concentration, reaching 4 g. per 100 ml. within 22 days in 10 patients (I to X, Table II). This rising albumin concentration corresponding to daily infusions has been plotted against time in Fig. 2, together with blood volume and differences of colloid osmotic pressure between plasma and ascitic fluid. When the infusions were continued at this rate the serum concentration rose further, and a value of 4·7 g. per 100 ml. was achieved on three occasions (patients I, II, and III). Maintenance dosage, usually of 50 g. per week, was then given whenever a satisfactory diuresis had occurred, and the serum concentration has been subsequently maintained in all instances between 3 g. and 4 g. per 100 ml. except in patient IX, who lost considerable quantities of protein in the urine (see above) and in patient X. During periods of maintenance of up to two and a half years in other successfully treated patients no spontaneous change in the serum albumin concentration has been apparent, although a drop from 4 to 3 g. per 100 ml. in patient V followed an increase in the intervals

## TABLE II

### *Protein Concentrations of Plasma and Ascitic Fluid*

The estimations were made on a day (*a*) before infusions commenced, (*b*) when the plasma-albumin concentration was at its maximum, and (*c*) at intervals after the completion of the initial course of infusions. Concentrations (g./100 ml.) are of albumin (*A*), combined α- and β-globulins (α+β), and γ-globulin (γ), in plasma and ascitic fluid.

**1. PLASMA**

| *Patient* | *I* | | | *II* | | | *III* | | | *IV* | | | *V* | | | *VI* | | | *VII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ |
| (*a*) Pre-infusion | 2·6 | 1·5 | 2·5 | 2·5 | 1·7 | 3·4 | 2·0 | 1·7 | 2·4 | 2·3 | 1·5 | 2·8 | 1·0 | 1·7 | 3·0 | 2·0 | 0·9 | 4·6 | 2·6 | 1·3 | 1·5 |
| (*b*) Maximum | 4·8 | 1·9 | 1·4 | 4·7 | 1·6 | 1·7 | 4·8 | 1·4 | 1·8 | 4·6 | 1·3 | 1·7 | 4·4 | 1·8 | 2·2 | 5·0 | 0·8 | 2·8 | 4·4 | 1·0 | 0·9 |
| (*c*) Post-infusion: | | | | | | | | | | | | | | | | | | | | | |
| 1 week | 4·0 | 1·2 | 1·7 | .. | .. | .. | .. | .. | .. | 4·3 | 1·3 | 2·0 | 4·3 | 0·8 | 2·8 | 3·6 | 1·2 | 3·6 | 3·2 | 1·7 | 1·5 |
| 1 month | 3·8 | 1·7 | 1·5 | .. | .. | .. | .. | .. | .. | 3·6 | 1·3 | 2·6 | 3·5 | 1·0 | 3·2 | 3·7 | 0·8 | 3·7 | 3·1 | 1·2 | 1·5 |
| 2 months | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3·5 | 1·4 | 2·3 | 3·0 | 0·9 | 2·6 | 2·9 | 1·9 | 3·4 | 3·0 | 1·2 | 1·2 |
| 6 months | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3·0 | 1·3 | 2·2 | 3·4 | 1·3 | 2·6 | 3·8 | 1·1 | 3·4 | .. | .. | .. |
| 12 months | .. | .. | .. | .. | .. | .. | .. | .. | .. | 4·1 | 1·2 | 1·6 | 4·2 | 1·0 | 3·2 | 3·0 | 1·3 | 2·5 | .. | .. | .. |
| 30 months | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 3·0 | 1·5 | 3·6 | 3·4 | 1·7 | 2·8 | .. | .. | .. |

**2. ASCITIC FLUID**

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Pre-infusion | 0·5 | 0·4 | 0·5 | 0·6 | 0·4 | 0·4 | 0·5 | 0·4 | 0·7 | 0·3 | 0·2 | 0·2 | 0·2 | 0·2 | 0·4 | 0·3 | 0·1 | 0·4 | (Not known) | | |
| (*b*) Maximum | 2·8 | 0·8 | 0·9 | 2·1 | 0·6 | 0·5 | 2·9 | 0·7 | 1·2 | 3·0 | 0·5 | 0·6 | 1·7 | 0·8 | 1·3 | 2·0 | 0·7 | 1·4 | 2·1 | 0·6 | 0·4 |

**1. PLASMA**

| *Patients* | *VIII* | | | *IX* | | | *X* | | | *XI* | | | *XII* | | | *XIII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ | A | α+β | γ |
| (*a*) Pre-infusion | 2·1 | 1·1 | 1·0 | 2·3 | 1·0 | 2·4 | 1·6 | 1·3 | 3·7 | 1·8 | 2·1 | 2·4 | 2·2 | 1·6 | 3·3 | (Not known) | | |
| (*b*) Maximum | 5·0 | 0·8 | 1·2 | 4·0 | 1·0 | 2·1 | 4·2 | 1·2 | 3·2 | 3·2 | 2·0 | 2·1 | .. | .. | .. | 2·9 | 1·6 | 1·3 |
| (*c*) Post-infusion: | | | | | | | | | | | | | | | | | | |
| 1 week | 2·8 | 1·1 | 1·6 | 2·8 | 1·6 | 1·6 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1 month | 4·0 | 1·2 | 1·6 | 3·0 | 1·4 | 1·8 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 2 months | 3·4 | 1·3 | 1·8 | 2·7 | 1·2 | 1·6 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 6 months | 3·0 | 1·2 | 1·5 | 2·5 | 1·3 | 2·1 | 2·3 | 1·5 | 3·1 | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 12 months | 3·8 | 0·5 | 1·4 | .. | .. | .. | 2·7 | 1·3 | 2·9 | .. | .. | .. | .. | .. | .. | .. | .. | .. |

**2. ASCITIC FLUID**

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Pre-infusion | .. | .. | .. | 0·5 | 0·1 | 0·4 | .. | .. | .. | 0·5 | 0·4 | 0·6 | 0·4 | 0·2 | 0·5 | (Not known) | | |
| (*b*) Maximum | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1·4 | 0·7 | 0·8 | .. | .. | .. | 1·0 | 0·3 | 0·4 |

## TABLE III

### *Colloid Osmotic Pressures*

(P = plasma; AF = ascitic fluid; D = difference between them.) The estimations were made (*a*) before albumin infusions and (*b*) at the maximum plasma albumin concentration.

| *Patient* | *I* | | | *II* | | | *III* | | | *IV* | | | *V* | | | *VI* | | | *VII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* |
| (*a*) Pre-infusion | 21 | 3·5 | 17·5 | 27 | .. | .. | 23 | 3 | 20 | 22 | 3 | 19 | 27 | 3 | 24 | 23 | 3 | 20 | 22 | .. | .. |
| (*b*) Maximum | 46·5 | 20 | 26·5 | 45 | .. | .. | 46 | 25 | 21 | 41 | 16 | 25 | 53 | 14 | 39 | 43 | 19 | 24 | 36 | 13 | 23 |

| *Patient* | *VIII* | | | *IX* | | | *X* | | | *XI* | | | *XII* | | | *XIII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* | *P* | *AF* | *D* |
| (*a*) Pre-infusion | .. | .. | .. | 17 | 2 | 15 | 18·5 | .. | .. | 24 | 4·5 | 19·5 | 21 | 3 | 18 | 27 | 4 | 23 |
| (*b*) Maximum | .. | .. | .. | .. | .. | .. | .. | .. | .. | 34 | 9 | 25 | .. | .. | .. | 32 | 6 | 26 |

between infusions from a week to a fortnight. The serum globulin concentration fell during the primary course of infusions (Table II) at the same time as the albumin concentration was rising. The $\gamma$-globulin level fell by at least 20 per cent. in seven of 11 patients. In five of these seven, subsequent figures are available: the concentration in two (patients VI and VII) had returned to the original level within a week of the cessation of daily infusions; in two others (patients I and IV) it had shown some return, and in patient IX (who had developed proteinuria) there was no evidence of a return to pre-infusion values. The plasma concentration of $\gamma$-globulin was followed further in two patients in whom it had not fallen during infusion: in patient V no significant change in concentration occurred at this stage or later in the period of maintenance, and in patient VIII a rise from 1·0 g. to 1·6 g. per 100 ml. was sustained for the remaining 12 months of his life.

These changes in the plasma were found to be reflected by changes in the composition of the ascitic fluid, but the relationship between the total protein and the albumin and globulin concentrations shows important differences. The rising albumin concentration in both fluids maintained between them a difference which, while varying from patient to patient, remained either constant or increased by not more than 1 g. per 100 ml. in any individual case. At the same time the total protein concentration of the ascitic fluid increased from an average pre-infusion value of 1·15 g. per 100 ml. to 4·05 g. per 100 ml. at the maximum, while the corresponding rise in the plasma was from 6·15 to 7·65 g. per 100 ml. This marked rise of protein concentration in the ascitic fluid was due mainly to an increase in the value for albumin, but the globulin also increased to a lesser degree. Although this occurred at a time of falling concentration of serum globulin and rapid changes in the albumin: globulin ([A]/[G]) ratio, one constant relationship was observed. The [A]/[G] ratio of the ascitic fluid, initially 13 per cent. higher than that of the plasma, maintained this difference in spite of considerable increases in both ratios (Fig. 3). The drop in globulin concentration in the plasma has been further analysed (Table IV), and changes in total intravascular $\gamma$-globulin compared with the changes in the ascitic fluid. In those patients in whom the ascitic fluid volume changed but little (patients I, II, III, and VI, and patients IV and VII before diuresis), there was a loss from the plasma of from 11 to 30 g. $\gamma$-globulin, and a gain in the ascitic fluid of about 20 to 40 g. (no preliminary value being available in patient VII). When the diuresis occurred in patients IV and VII, reabsorption of the ascites and its contained protein was accompanied by an approximately equivalent rise in the total plasma $\gamma$-globulin. There is a significant negative correlation between these two changes. In patient V, an increase of 43 g. $\gamma$-globulin in the plasma was accompanied by the disappearance of ascites estimated to have contained 20 g. (in this period the patient received three pints of blood by transfusion, which would add an additional 10 g. of $\gamma$-globulin to the plasma). It appears probable that these changes in $\gamma$-globulin concentration in both plasma and ascitic fluid are simply the results of shifts of the protein between these body compartments.

The amount of albumin needed to raise the serum concentration to normal (Table Ib) is much greater than was expected from calculations based on total circulating albumin, the average quantity infused in each complete course being 1,120 g. The urinary excretion of nitrogen was studied in patients I, V, and VIII during the whole period of daily infusions (the oral intake being kept

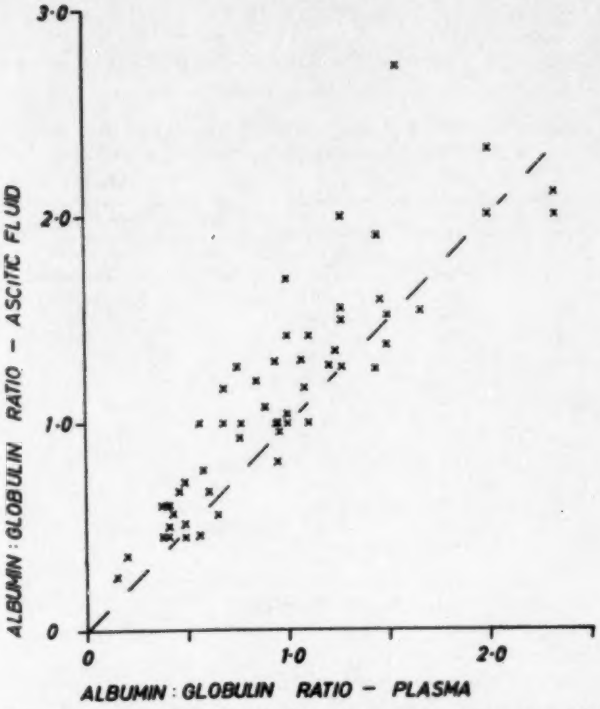


FIG. 3. Albumin/globulin ([A]/[G]) ratios of plasma and ascitic fluid samples taken simultaneously. Comparisons are shown for all patients except Nos. VIII and X, and include values at varying serum albumin concentration except for patients VII, IX, XII, and XIII. The line [A]/[G] plasma = [A]/[G] ascitic fluid has been drawn, and it is seen that most points lie above it. The mean regression (assuming errors in the two parameters to be equal) gives the formula [A]/[G] AF = 0·03+1·13([A]/[G]) P. (AF = ascitic fluid: P = plasma.)

approximately constant), and was found during this limited period to be greater than its excretion in a pre-infusion period of equal duration by 20, 50, and 45 g. nitrogen respectively, the equivalent of 120, 300, and 270 g. of protein. Allowing for this loss, the quantity of albumin infused was still approximately three times greater than expected. Further evidence along these lines has been sought by the use of isotope-labelled albumin, and rates of protein catabolism have been studied at different levels of serum concentration in patients IV, VI, and X. Two different techniques have been used. In patient VI the distribution and

catabolism of a single dose of $^{131}$I-labelled albumin was studied just before albumin infusions, when the serum-albumin concentration was 2 g. per 100 ml., and then again three months later, when the serum level in the period of maintenance therapy was 3·9 g. per 100 ml. As can be seen from Table V, analysis of the derived curves reveals an increase in the previously depressed rate of

TABLE IV

*Changes in γ-Globulin Content of Plasma and Ascitic Fluid (g.) during the Initial Course of Albumin Infusions*

Ascitic fluid volumes were either measured directly or deduced from paracentesis to dryness and other data (e.g. girth, body-weight changes).

| | *Plasma* | | | *Ascitic fluid* | | |
|---|---|---|---|---|---|---|
| | *Total γ-globulin* | | | *Total γ-globulin* | | |
| *Patient* | *pre-infusion* | *post-infusion* | *Change from pre-infusion value* | *pre-infusion* | *post-infusion* | *Change from pre-infusion value* |
| I | 75 | 56 | —19 | 6 | 36 | +30 |
| II | 88 | 58 | —30 | 40 | 80 | +40 |
| III | 84 | 64 | —20 | 56 | 96 | +40 |
| IV | 126 | (*a*) 98 | —28 | 10 | (*a*) 40 | +30 |
| | | (*b*) 105 | —21 | | (*b*) 30 | +20 |
| | | (*c*) 126 | 0 | | (*c*) 0 | —10 |
| V | 74 | 117 | +43 | 20 | 0 | —20 |
| VI | 140 | 163 | +23 | 30 | 0 | —30 |
| VII | 54 | (*a*) 43 | —11 | Less than 4 | 24 | +(20—24) |
| | | (*b*) 72 | +18 | | | 0—(—4) |
| VIII | 40 | 56 | +16 | No measurable ascites | | |
| IX | 115 | 94 | —21 | | | |
| X | 157 | 163 | + 6 | | | |

(*a*) = Commencement of diuresis.
(*b*) = Height of diuresis.
(*c*) = Completion of diuresis.

breakdown proportionately greater even than the rise in serum-albumin concentration. In patients IV and X only one turnover study was carried out, but it was immediately followed by albumin infusions at the rate of 50 g. per day, and measurements of urine and plasma activity were repeated during a four-day gap in the infusions, when the serum albumin had returned to normal. Calculations based on the total urinary radioactivity and the specific activity of the serum albumin indicate a very great increase in the catabolic rate of albumin, again proportionately much larger than the rise in serum concentration. The closest arithmetical relationship between the different factors in the three patients is that the quantity of albumin catabolized per day is proportional to the square of the total circulating albumin. Deductions as to quantities of protein catabolized during the period of infusions, when the plasma activity is changing rapidly, can be only approximate, but the figures derived are similar to those obtained from urinary nitrogen values as described above.

2. *Changes in colloid osmotic pressure (COP) in serum and ascitic fluids.* Multiple COP measurements have been made in the serum of patients studied,

and have been plotted in Fig. 4. The initial serum COP ranged from 17 to 27 cm. of water (Table III), the two lowest readings (patients IX and X) being obtained from patients with massive oedema and only a trace of ascites, whereas the group with predominant ascites had values which lay between 21 and 26·5 cm. of water. Ascitic fluid COP before treatment was always less than

TABLE V

*Changes in Rate of Albumin Catabolism following Intravenous Infusions and Elevation of Serum Concentration*

Catabolic rate measured by the urinary excretion of radioactivity from injected $^{131}$I-labelled albumin.

| *Patient* | | *Serum albumin (g./100 ml.)* | *Total circulating albumin (g.)* | *Quantity catabolized per day (g.)* | *Radioactivity appearing in urine per day as percentage of total plasma activity (U/P)* |
|---|---|---|---|---|---|
| IV | Before infusion | 2·3 | 103 | 4·6 | 5 |
| | After infusion | 4·6 | 253 | 34·0 | 13 |
| VI | Before infusion | 2·0 | 66 | 3·3 | 5 |
| | After infusion | 3·9 | 156 | 10·9 | 7 |
| X | Before infusion | 1·6 | 69 | 2·0 | 3 |
| | After infusion | 3·8 | 206 | 16·8 | 11 |

5 cm. of water, reflecting its low protein concentration, and the COP difference between serum and ascitic fluid varied from 15 to 24 cm. of water. These values were followed during the course of infusions in seven instances (patient VII, who had only oedema at the commencement of infusions, developed ascites as the treatment progressed (Fig. 1); the fluid then disappeared, but reappeared in small quantities three months later, when further measurements were possible). The response of the serum COP to albumin infusions closely followed the albumin concentration and, at the completion of the full courses of treatment (patients I to X), had risen into the normal range, values now lying between 36 and 53 cm. of water (Table III). The ascitic fluid showed a similar response, and the COP rose in concert in all instances (Fig. 4), the highest readings varying from 13 to 25 cm. of water (Table III). The difference between values in the two fluids has been widely discussed as the 'effective portal pressure' (Giges and Kunkel, 1954; James, 1949), and here showed a very variable response. In patient III alone there was no significant increase in this difference, but in the other five in whom pre-infusion figures are available (patients I, IV, V, VI, and XI) there was a variable increase by amounts of up to 15 cm. of water. It was possible to check these measurements three days after the last infusion in four of these five patients, as traces of the disappearing ascitic fluid have tended to remain for a week or two longer, and the two measurements (and therefore also the difference between them) have remained constant. After this interval equilibrium may be assumed to have taken place (Mankin and Lowell, 1948).

3. *Changes in blood volume.* Plasma volume measured before infusions commenced ranged from 39 to 63 ml. per kg. of body-weight (ascites- and oedema-free) (average 54 ml. per kg.), and the red-cell mass from 1·4 to 2·6 l. (average 1·9 l.). Anaemia was to some extent responsible for supranormal plasma volumes, the total blood volume ranging from 67 ml. to 95 ml. per kg., or

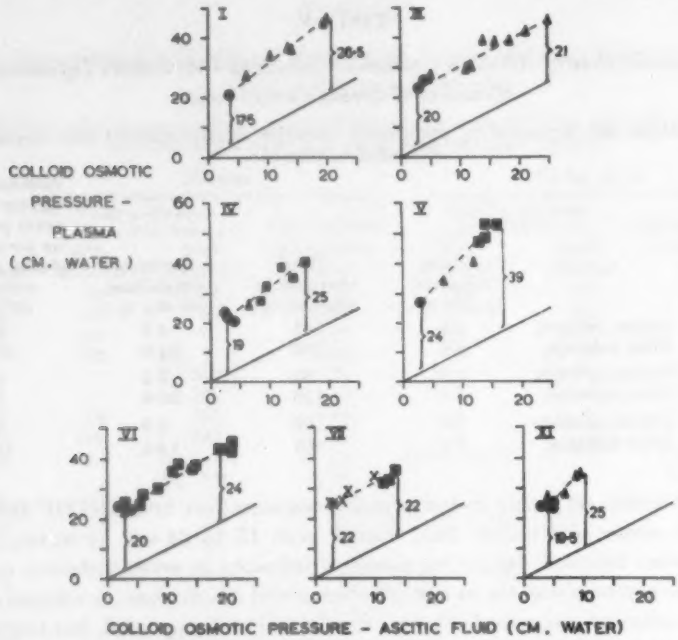


FIG. 4. Colloid osmotic pressure (COP) of plasma and ascitic fluid samples taken simultaneously both before therapy (●), and during the period of daily infusions, before (▲) and after (■) diuresis had commenced; in patient VII values recorded at a later date, when ascites was recurring, are also included (X). The line COP plasma = COP ascitic fluid has been drawn in each case (continuous line); also a line through experimental values (best eye fit—discontinuous line). Numerals represent the mean values of plasma-ascitic fluid COP difference before infusions commenced and at maximum. The increased COP difference, seen to some extent in each patient studied, is reflected in the increasing steepness of the lines drawn through experimental values, and is most marked in patient V.

900 ml. to 1,510 ml. per cu. m. of height cubed. After infusions commenced there was a drop in venous haematocrit level of at least 5 per cent. in seven patients and, when the plasma volume was again estimated on six occasions in three patients, the degree of expansion as calculated from the haematocrit was confirmed to within ±10 per cent. Patient IV lost 500 ml. of blood in the week before infusions began, rendering the haematocrit level unreliable as an index of blood volume, and so plasma-volume studies were repeated at the conclusion of the period of daily infusions. There was no evidence of significant loss of blood in any other patient during the period of study. The calculated changes

in total blood volume accompanying the transfusions have been charted in Fig. 2, where the highest values attained are seen to reach 130 ml. per kg. Patients III and IX showed no significant change in day-to-day venous haematocrit measurements; in patient IX, however, when serial haematocrit measurements were taken in the 24-hour period following the infusion of 200 ml. of 25 per cent. albumin solution (the infusion lasting one and a half hours), the maximum fall was seen approximately two hours after the infusion finished, and virtually disappeared three hours later. If the infused protein were assumed to draw fluid from the extracellular space so as to dilute itself to the concentration of serum proteins, then the expansion calculated from the fall in haematocrit level was the same as that expected from the quantity of albumin infused. The degree of expansion bore no relationship to the success or otherwise of the treatment, but it followed quite closely the degree of increase in the COP difference. This relationship can be seen in Fig. 2, together with the time-relationship to the rising albumin concentration. The possible implications of these interrelationships are discussed below.

## *Discussion*

### A. *Clinical*

It is necessary in treating patients with chronic disease to aim at restoring them to their previous well-being and daily life, whether or not it is possible to reverse the pathological process or remove all manifestations of the disease. Even when the remaining years of life are limited in number, the removal of invalidism from the terminal period is of great importance to patient and relatives alike. Cirrhosis of the liver, resulting in ascites and oedema which have become resistant to diuretic therapy, constitutes a problem which is generally regarded as being without solution, and the patient is left not only with a very limited life expectancy, but with the two major incapacities of fluid retention and marked loss of well-being. These two clinical features are the chief cause of invalidism in this group of patients, and both must be significantly improved to enable the patient to return to an active life.

*Effects of courses of daily albumin infusions.* Kunkel, Labby, Ahrens, Shank, and Hoagland (1948) and Post, Rose, and Shore (1951) have demonstrated that most of these patients may be benefited by the use of albumin infusions, and many restored to a virtually normal life for limited periods. The results in the present small series confirm these findings in approximately the same proportion of cases as has been recorded previously. Of 13 patients, the three who died of complications of the disease before a full course of treatment was given are not considered to have deteriorated as a result of the infusions, and so are not available for assessment of the success rate. Seven of the remaining 10 have been restored to their normal life; two of these patients are alive, and still receiving maintenance infusions, at intervals of 15 and 32 months from the time of presentation. The detection of those patients suffering from refractory fluid retention due to cirrhosis of the liver who are likely to respond to albumin infusions has not here been clearly defined, but the previously recorded fact

(Faloon, Eckhardt, Murphy, Cooper, and Davidson, 1949) that oedema is more susceptible to treatment than ascites has been confirmed. The resistance of ascites is the principal problem with this form of therapy, and it is important to note that in patients IV and VII diminution in girth did not occur until the serum albumin concentration was greater than 4 g. per 100 ml. Unfortunately it has not been possible to point to any other preliminary finding, before treatment commenced, which might enable an accurate prediction of the result of therapy to be made, but two facts are worthy of comment. First, a serum sodium concentration of less than 130 m-equiv. per litre was seen in all the patients who did not respond, but in only one who did; and secondly, a protein concentration of 1 g. per 100 ml. in the ascitic fluid before treatment clearly divided those in whom a complete diuresis was seen (less than 1 g. per 100 ml.) from those who were resistant to therapy (greater than 1 g. per 100 ml.). The exact significance of these findings is not entirely clear.

Relief from the symptoms of malaise, lethargy, and anorexia is as important for the return of the patient to his environment as is the removal of retained fluid, and was fully achieved in all patients at the completion of therapy. As this effect followed the first few infusions, and occurred whether or not diuresis was to take place, it cannot have been a direct consequence of the elimination of the ascites and oedema. It is of note that the only two patients who did not suffer from these symptoms, and therefore noticed no improvement in well-being, were also the only two in whom no expansion of blood volume was observed during the course of therapy. It is probable that these symptoms and their relief are in part connected with alterations in blood volume.

*Effects of maintenance infusions.* There is only one previously recorded patient (Post, Rose, and Shore, 1951) who received maintenance infusions (reported as alive at the end of 12 months), and the usual plan adopted by earlier workers for the use of intravenous albumin has been a short course of daily infusions, followed on discharge from hospital by the use of salt restriction and diuretics aimed at preventing reaccumulation of ascites; alternatively, repeated 'booster dose' courses of daily infusions have been given each time the fluid returned. In the present series the withdrawal of infusions has not been attempted, but lengthening the interval between them from one to two weeks in two patients was followed by a drop in serum albumin concentration, suggesting that rates of synthesis had not improved. Maintenance infusions have, therefore, been necessary to keep the serum albumin concentration at or near normal levels in every patient in whom a complete diuresis has occurred. Other evidence of improvement in liver-cell function was not sufficiently frequent in this small group of patients for any firm conclusions to be drawn, but it was encouraging to note the disappearance of attacks of portal-systemic encephalopathy in two patients, the lowering of a raised serum bilirubin level during maintenance therapy in a third, and a return to normal of prothrombin activity in two others. It appears likely that the return of the total albumin pool to normal improved the function of some enzyme systems in ways which are not yet understood.

*Complications during therapy.* Complications are expected in a disease such as cirrhosis of the liver, particularly when it has reached the degree of severity of the group here studied, and it would be surprising if some were not seen during the total of 128 patient-months which are included in this study. On the other hand, when using a plasma expander in a condition in which an increased blood volume (Perera, 1946) and a tendency to haemorrhage are present, all incidents occurring during the course of therapy must necessarily be considered as possible sequelae; ideally the patients should be compared with a similar group of patients with severe cirrhosis in which the treatment was not given. The gravity of the two fatal pyrogenic reactions was very disturbing, and illustrates the extreme danger of febrile reactions generally in these patients. The cause is believed to be the infusion of pyrogen as a result of bacterial contamination; and as this is a preventable complication, not due to the albumin *per se*, it forms the subject of a separate report (Dykes, 1961). The temporary increases in serum bilirubin were of no serious importance, and always appeared and subsided within a few days of an infusion; they cannot, therefore, have any connexion with homologous serum jaundice. The serious degree of proteinuria seen in patient IX has not been adequately explained, and its relationship to the albumin infusions remains indefinite. There is no doubt that it appeared suddenly within two weeks of the commencement of infusions, and there was an associated haematuria, neither being present before infusions started. There were, however, no other manifestations of an allergic phenomenon, and, as the serum complement did not decrease significantly, it is unlikely that albumin had induced acute nephritis (Ellis and Walton, 1958). The diabetes, in the absence of significant histological findings in the glomeruli, appears unlikely to be the explanation, and renal vein thromboses were not found, although carefully sought. The presence of renal histological abnormalities has been noted in association with cirrhosis by Fisher and Hellstrom (1959), and the changes observed in this patient may be a further manifestation of this combination of lesions.

Gastrointestinal haemorrhage is a common complication of cirrhosis of the liver, and is the result of portal hypertension (Hunt, 1958), an increased incidence of peptic ulceration (Lipp and Lipsitz, 1952), an increased bleeding tendency due to deficiency of prothrombin, fibrinogen, or platelets, and increased capillary fragility (Sherlock, 1955). Several earlier investigators (Faloon, Eckhardt, Murphy, Cooper, and Davidson, 1949; Havens and Bluemle, 1950) have reported catastrophic haemorrhage in a number of patients treated with albumin infusions, although the incidence has varied from series to series. Of the three such instances in the present series, one was the result of a very large gastric ulcer, and it is felt that albumin infusions played no part in its causation. Of the other two, that occurring in patient XI must be considered as a possible consequence of therapy, but, as this patient had suffered 11 previous haemorrhages, this is by no means certain. The third instance (in patient III) occurred 24 hours after one of the few infusions given of 100 g. albumin, but this patient had shown no permanent blood-volume expansion (Fig. 2). As the

incidence of haemorrhage reported by previous workers has tended to be higher when using a dose of 100 g., it seems a wise precaution to limit each infusion to 50 g.

It seems that the beneficial clinical results described above were not vitiated by an undue incidence of complications which could fairly be attributed to the albumin therapy in such seriously ill patients. Whether life has 'on average' been prolonged in this series of patients is uncertain, and could be proved only in future studies with matched controls. On clinical grounds two patients (V and VIII) were regarded when first seen as unlikely to live for longer than six months; after albumin infusions they both returned to good health, and remained free from ascites and oedema for two and a half years and one year respectively.

B. *Biochemical*

*Changes in protein concentrations; unexpectedly large amounts of albumin necessary; shift of globulins to the extravascular compartment.* Infusion of human albumin would be expected to raise the plasma concentration, and in the present series there is no reason to suppose that, if treatment had been carried on for long enough (in the absence of significant urinary loss), it would not have been possible to achieve supranormal levels in every patient. The very large amount of albumin necessary to raise the plasma concentration to normal was surprising, although other workers have previously recorded similar experience. It was expected that the quantity normally present in the total exchangeable albumin pool, 200 to 400 g., a figure derived from studies with labelled albumin (Berson, Yalow, Schreiber, and Post, 1953), would be close to the maximum amount needed to return the serum concentration to normal; yet it was often necessary to give three or four times this calculated value in the initial course. Urinary nitrogen figures available from three of the patients showed some increase, but equivalent to only about a quarter of the total unaccounted for; it is not, therefore, possible that the protein had been catabolized and excreted, although it is conceivable that catabolism releases products of which the tissues are deficient, and which may then be immediately incorporated into resynthesized protein. To further the solution of this problem, information was obtained by measuring excretion of radioactive iodide after the injection of a dose of $^{131}$I-labelled albumin at the time daily infusions began, as it is known that $^{131}$I-labelled amino acids and free $^{131}$I catabolic products of the albumin are excreted in the urine within four days without resynthesis to protein (Cohen, Holloway, Matthews, and McFarlane, 1956). The iodine-labelled amino acid is apparently 'recognized' as unusual, and the iodine atom removed and excreted. Values of the catabolic rate of albumin obtained by this method largely agree with the results derived from the studies of nitrogen excretion, and this fact suggests that, if catabolism of the unaccounted albumin occurs to any extent, it does not proceed to the level of amino acid which would have produced corresponding urinary radioactivity. It must be emphasized that the albumin used for infusions was obtained from the same source as that which was labelled

with $^{131}I$, and these results may therefore be interpreted as representing fractional breakdown rates not only of the original labelled material, but also of the protein which was later infused in massive doses. Fractional rates of breakdown in the first two days after injection of the tracer were not higher than in subsequent days by more than 1 per cent. of the dose, suggesting that the protein was of very satisfactory quality (McFarlane, 1956). It may, therefore, be concluded that the very large amounts of albumin administered in excess of what was anticipated to be necessary were either incorporated intact in the tissues, or that, if catabolized at all, they were broken down to a stage short of the production of free amino acid. Further studies have shown that the increased rate of catabolism encountered when the plasma albumin concentration returned to normal was maintained as long as maintenance infusions were continued, confirming the conclusion reached above that there was no improvement in the powers of protein synthesis.

The fall in plasma $\gamma$-globulin concentration, judged from the analyses of total circulating and total ascitic-fluid $\gamma$-globulin (Table III), is apparently due simply to shifts in quantities of the protein between the two compartments, and no evidence has been obtained of a change in its rate of catabolism. It has previously been shown that albumin infusions may alter concentrations of cholesterol and cholinesterase in nephrotic patients (Soothill and Kark, 1956), but the possibility of shifts such as those described here must be carefully examined before these changes in concentration are ascribed to alterations in anabolic rates. The view has also been advanced (Gray and Barron, 1943) that the elevated $\gamma$-globulin concentrations in hypoalbuminaemic patients may be due to an attempt to maintain normal colloid osmotic pressure; but the persistence of markedly raised $\gamma$-globulin levels months after the serum-albumin concentration has been permanently restored to normal strongly argues against such a hypothesis.

*Relationship between albumin/globulin* ($[A]/[G]$) *ratios of plasma and ascitic fluid.* The rising protein concentration of the ascitic fluid has been noted by earlier workers (Patek, Mankin, Colcher, Lowell, and Earle, 1948), but the relationships between the [A]/[G] ratios in plasma and ascitic fluid have not previously been studied in any detail. It is known that the [A]/[G] ratio of serous transudates and oedema fluid is higher than that of the plasma (Freeman and Joekes, 1956) and this fact is here confirmed. It is also now shown that a change in this ratio in the plasma is rapidly followed by a similar change in the ascitic fluid, such that [A]/[G]-ascitic fluid $= K$ [A]/[G]-plasma, where, in the present series, $K = 1{\cdot}13$ on average. This direct dependence of the ascitic-fluid [A]/[G] ratio on that of the plasma during the period of rapid change in albumin concentration confirms the rapid transfer of protein molecules between the two compartments already shown by radioactive labelling techniques (Bauer, Blahd, Fields, and Getchell, 1954). The increase in total ascitic-fluid globulins, seen whenever their concentration was determined at the time of rising serum albumin concentration, implies an imbalance between the rate of loss from the plasma on the one hand and the rate of reabsorption from the

ascitic fluid on the other. During this period the concentration of plasma globulin (unlike the ascitic-fluid globulin) has fallen markedly, and if the movement of protein from plasma to ascitic fluid is governed by laws either of diffusion or of filtration, then the rate of this transfer must also diminish; it follows that the increase of total ascitic-fluid globulin can be due only to reduced reabsorption. The only major factor likely to alter the reabsorptive process was the greatly increased albumin concentration of the ascitic fluid, and it therefore seems probable that reabsorption is governed by direct competition between the protein molecules. It might, however, be suggested that the different ratios are the result of the removal of one of the globulins selectively from the ascitic fluid (for example, by the reticulo-endothelial system); but when the relative [A]/[G] ratios are re-determined for each of the electrophoretically separate globulins, $K$ is found to be 1·3 ($\alpha_1$-globulin), 1·5 ($\gamma$-globulin), 1·25 ($\beta$-globulin), and 1·8 ($\alpha_2$-globulin). It is apparent that albumin is present in a proportionately higher ratio to each of the globulins in the ascitic fluid than in the plasma, and that the difference is not due to removal of any one of them selectively. A further possibility might be that newly made albumin is released from the liver into the peritoneal cavity when albumin catabolism is occurring from the plasma. The present writer has found, however, that intravenous injections of tracer doses of $^{131}$I-labelled albumin result, after a period of equilibrium between the different compartments, in a permanently higher specific activity in the ascitic fluid than in the plasma, and the newly formed albumin (which has no radioactivity) must therefore be delivered predominantly into the blood. Other conceivable explanations having been excluded, the constant relationship between the [A]/[G] ratios of plasma and ascitic fluid is best explained in terms of a selective passage of protein through the capillary walls, with non-selective (that is, competitive) lymphatic reabsorption of all proteins. The capillary selection is presumably dependent principally on the molecular weights of the various globulins, and the different ratios for the electrophoretically separated globulins accord with this idea, assuming that the $\beta$-globulin in the ascitic fluid is principally siderophilin (molecular weight 90,000). The relative ratios might have been expected to be lower for $\alpha_1$-globulin (its principal component normally having a molecular weight which is less than that of albumin); but this fraction consists of a number of proteins which are possibly altered in relative amounts in cirrhosis.

*Relative colloid osmotic pressure (COP) of plasma and ascitic fluid.* The close interdependence of the [A]/[G] ratios of plasma and ascitic fluid having been demonstrated, it remains to consider the relationship between the respective total protein concentrations. Starling (1895–6) held the view that there was a balance between the difference in hydrostatic pressures on the one hand and the COPs on the other, but Cherrick, Kerr, Read, and Sherlock (1960), attempting to confirm this view by measuring hydrostatic and colloid osmotic pressures in plasma and ascitic fluid, have been unable to demonstrate a close balance of forces. The use of portal pressure as an approximation to the capillary pressure involved is not, however, entirely valid; the latter must be the mean of a

number of widely different values, probably including splanchnic, hepatic, and systemic capillary pressures, and may well be affected by such factors as the rate of flow through and out of the capillary and the presence of interstitial oedema. Starling's hypothesis therefore lacks supporting evidence of a direct nature, and must be tested in other ways to assess its validity. The present type of study seems to promise the information required, in that the response of all four factors to the variation of one (plasma COP) may be observed; Mankin and Lowell (1948) reported three such studies, in which the COP difference remained constant throughout. The evidence of the present investigation, however, appears at first sight to contradict their finding, since in all cases there has been an increase in the COP difference, varying in amount from patient to patient (Fig. 4). On the occasions when this was checked there was no change in the ascitic-fluid hydrostatic pressure, and it remains to be considered whether there was an increase in mean capillary pressure in the patients who showed the greatest increase in COP difference. Bayliss and Starling (1894), working on dogs, showed that rapid infusions of saline produced a considerable increase in systemic and portal venous pressures, which returned to normal 10 to 15 minutes after the cessation of infusions, as the infused fluid left the circulation. Subsequent workers have confirmed this effect on systemic venous pressure, and have demonstrated a marked rise in cardiac output, with virtually no change in pulse rate or systemic arterial pressure (Warren, Brannon, Weens, and Stead, 1948). It is highly probable that capillary pressure follows venous pressure to some extent, to maintain an even gradient from the arteriole to the large vein. The present observations have not, unfortunately, been extended to cover prolonged periods of blood-volume expansion, but it has been a common finding that slight distension of the neck veins, produced within a few days of commencing daily infusions, persisted until the patient's discharge from hospital. The degree of correlation demonstrated in Fig. 2 between COP difference and blood volume does, therefore, appear to be of great significance, even though the above assumptions must be liable to considerable variability. The manner in which the greatest and least expansions in blood volume on the one hand correspond with the greatest and least increases of the plasma/ascitic-fluid COP difference on the other (patients V and III respectively) argues strongly in favour of Starling's balance which, when disturbed in one direction, is compensated for in another. It seems logical to conclude that the hypothesis is always true in cirrhotic ascites when the plasma hydrostatic pressure is interpreted as defined above.

*Blood volume.* A supranormal blood volume has been demonstrated previously in a significant proportion of patients with cirrhosis of the liver, together with other manifestations of a hyperdynamic circulation—warm hands, distended neck veins, and a high cardiac output (Murray, Dawson, and Sherlock, 1958). The standard previously used for comparison between patients has been body weight and surface area, but patients with cirrhosis are often considerably wasted and, as has been suggested by Allen, Peng, Chen, Huang, Chang, and Fang (1956), the cube of the height may be a more satisfactory basis of

comparison. When the values from normal subjects published by Hicks, Hope, Turnbull, and Verel (1956) were re-plotted as the logarithm of blood volume (litres) against the logarithm of height (metres), the relationship was seen to be linear, with a slope of three in one, yielding the formula Bl. Vol. = 0·95 $H^3$ ($\pm$10%) (Squire, 1960). The values obtained for blood volume in the present group of cirrhotic patients before infusions are also normal or elevated, in relation both to body weight and to height cubed; that is, the supranormal volume is not merely relative to a reduced body weight. In spite of these supranormal values for blood volume, further expansion was seen during therapy in a number of patients (Fig. 2), whether or not a diuresis had taken place. The only limiting factor to the degree of expansion appeared to be the onset of the diuresis, after which the volume either contracted slightly or remained constant. Hence the optimum total blood volume in these patients appears to be set at some greater-than-normal value, and expansion to this extent should result in the release of sodium into the urine. The limited diuresis seen after each infusion in patient III, when the serum albumin had returned to normal, supports this contention, as diuresis always ceased when the volume increase resulting from each infusion had disappeared, that is, within 24 hours. Conversely, hypoproteinaemia eventually results in oligaemia (Squire, Blainey, and Hardwicke, 1957) and sodium retention, and it is probable that severe reduction in serum albumin and COP in the patients studied had already contracted the blood volume well below their optimum level. It may well be that the increased concentration of aldosterone seen in the urine of patients with ascites is the result of a failure of the diseased liver to conjugate and excrete the hormone at a normal rate. If this is true, one would expect avid sodium retention and a greater-than-normal blood volume, but the rapid onset of diuresis seen in some patients after albumin infusions shows that the sodium-retaining effect on the kidneys is still subject to physiological stimulus.

*Mechanism of diuresis*

1. *Oedema.* Transfer of fluid from the retained oedema to the blood-stream, and thence to the urine, occurred quite rapidly once the serum COP had been raised above the critical level (approximately 20 cm. of water); this is as expected from Starling's osmotic balance, assuming there was no major increase in the protein concentration of the interstitial fluid. This part of the diuresis, therefore, is very similar to that seen in cases of the nephrotic syndrome (Squire, Blainey, and Hardwicke, 1957) and, apart from the direct pressure from a peritoneal cavity tense with ascites on diminishing venous and lymphatic drainage of the legs, there is no reason to believe that oedema due to cirrhosis of the liver is in any way different in its aetiology from that seen in other hypoproteinaemic states.

2. *Ascites.* The situation in the ascitic fluid, however, is more complex, as the protein concentration rises with that of the serum and, if an osmotic balance continues to apply, elimination of the fluid is dependent on the removal of its

contained protein. It is likely that the rate of protein removal from the ascitic fluid by lymphatic action is proportional to its concentration, and that, in consequence, removal of water by this route will occur at a constant rate regardless of protein concentration. Transfer of protein from plasma to ascitic fluid, however, is probably by diffusion, or possibly by filtration, and the rate of exchange due to these two processes would be proportional, with diffusion, to the difference in concentrations between the two fluids, and, with filtration, to the plasma concentration itself. In any state of equilibrium the rate at which protein enters the ascitic fluid must equal the rate at which it leaves, and if these governing laws apply they may be formulated as follows:

A. *For diffusion from capillaries*:

Protein transfer into the ascitic fluid $= k_1([P]—[A])$

„ „ out of „ „ „ $= k_2[A]$

In equilibrium $k_1([P]—[A]) = k_2[A]$

or $$\frac{[P]—[A]}{[A]} = \frac{k_2}{k_1} = K \qquad (1)$$

B. *For filtration from capillaries*:

Protein transfer into the ascitic fluid $= k_3[P]$

„ „ out of „ „ „ $= k_4[A]$

In equilibrium $k_3[P] = k_4[A]$

or $$\frac{[P]}{[A]} = \frac{k_4}{k_3} = K^1 \qquad (2)$$

where [P] = protein concentration of plasma

[A] = „ „ „ ascitic fluid

and $k_1$, $k_2$, $k_3$, $k_4$, $K$, and $K^1$ are constants.

Study of the values for $K$ and $K^1$, as in Table VI, reveals that both have been markedly reduced on all occasions where further observations were possible after albumin infusions, whether total protein or albumin concentrations or COP measurements were used as the index of measurement (it is probable that COP, representing molecular concentration, is the most valid). The balance has therefore been altered in all cases in favour of net protein reabsorption from the ascitic fluid, but the step from this theoretical improvement to a practical disappearance of ascites depends on the governing factors of the constants. Of these the most important are probably capillary permeability, intracapillary hydrostatic pressure, and the effectiveness of the lymphatic system. It has already been suggested that the capillary pressure rises to a varying degree in all cases during the course of infusions, and this increase may influence the balance unfavourably on two counts: first, by increasing the rate of 'leakage' from the capillary, it would increase $k_1$ (or $k_3$), and secondly, by widening the COP difference, it would tend to decrease $k_2$ [A] (or $k_4$[A]). Study of Table III, however, reveals that the greatest increase in COP difference (patient V) occurred in association with a very successful diuresis, whereas the smallest

## TABLE VI

### *Protein Equilibrium Constants*

$K$ and $K^1$ (page 321) determined from total protein (TP) and albumin (A) concentrations, and colloid osmotic pressure measurements (COP) (*a*) before infusions commenced and (*b*) at maximum plasma albumin concentration.

$K$ = Ratio: Plasma minus ascitic fluid to ascitic fluid: [P]—[A]/[A].

$K^1$ = Ratio: Plasma to ascitic fluid: [P]/[A].

| *Patient* | *I* | | | *II* | | | *III* | | | *IV* | | | *V* | | | *VI* | | | *VII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Constant derived from | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* |
| $K$: | | | | | | | | | | | | | | | | | | | | | |
| (*a*) Pre-infusion | 3·7 | 4·2 | 5·0 | 4·4 | 3·2 | .. | 2·8 | 3·0 | 6·7 | 8·4 | 6·7 | 6·3 | 6·1 | 4·0 | 8·0 | 8·4 | 5·7 | 6·7 | .. | .. | .. |
| (*b*) Maximum | 0·8 | 0·7 | 1·3 | 1·5 | 1·2 | .. | 0·7 | 0·7 | 0·8 | 0·9 | 0·5 | 1·6 | 1·2 | 1·6 | 2·8 | 0·7 | 0·7 | 0·8 | 1·0 | 1·1 | 1·8 |
| $K^1$: | | | | | | | | | | | | | | | | | | | | | |
| (*a*) Pre-infusion | 4·7 | 5·2 | 6·0 | 5·4 | 4·1 | .. | 3·8 | 4·0 | 7·7 | 9·4 | 7·7 | 7·3 | 6·1 | 5·0 | 9·0 | 9·4 | 6·7 | 7·7 | .. | .. | .. |
| (*b*) Maximum | 1·6 | 1·7 | 2·3 | 2·5 | 2·2 | .. | 1·7 | 1·7 | 1·8 | 1·9 | 1·5 | 2·6 | 2·2 | 2·6 | 3·8 | 1·7 | 1·7 | 2·3 | 2·1 | 2·1 | 2·8 |

| *Patient* | *VIII* | | | *IX* | | | *X* | | | *XI* | | | *XII* | | | *XIII* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Constant derived from | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* | *TP* | *A* | *COP* |
| $K$: | | | | | | | | | | | | | | | | | | |
| (*a*) Pre-infusion | .. | .. | .. | 4·7 | 3·6 | 7·5 | .. | .. | .. | 3·2 | 2·6 | 4·3 | 5·5 | 4·5 | 6·0 | .. | .. | 5·7 |
| (*b*) Maximum | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1·5 | 1·3 | 2·6 | .. | .. | .. | 2·4 | 1·9 | 4·3 |
| $K^1$: | | | | | | | | | | | | | | | | | | |
| (*a*) Pre-infusion | .. | .. | .. | 5·7 | 4·7 | 8·5 | .. | .. | .. | 4·2 | 3·6 | 5·3 | 6·5 | 5·5 | 7·0 | .. | .. | 6·7 |
| (*b*) Maximum | .. | .. | .. | .. | .. | .. | .. | .. | .. | 2·5 | 2·3 | 3·8 | .. | .. | .. | 3·4 | 2·9 | 5·3 |

change (patient III) accompanied a therapeutic failure. It cannot, therefore, be a rise in portal pressure which prevents the occurrence of a diuresis in some patients. It also seems unlikely that further changes would occur in capillary permeability during the course of infusions, although it has been shown in a subsequent study by the author that before treatment has commenced there is occasionally a greatly increased rate of 'leakage' from plasma to ascitic fluid. Large numbers of lymphatic vessels have been described running adjacent to the portal vein whenever ascites is present (Theron and Allan, 1955; Baggenstoss and Cain, 1957), suggesting that the rate of lymph-flow from the liver is greatly increased. It has already been suggested by Gray (1951) that this increase in flow of lymph from the liver occurs as a result of the impedance to the hepatic venous outflow (Madden, Loré, Gerold, and Ravid, 1954), and results in a release of lymph into the peritoneal cavity. The further attainment of a maximum rate of protein withdrawal by the lymphatics of the peritoneum would then result in the accumulation of ascites, and attempts in these circumstances to improve withdrawal of ascitic fluid by raising its protein concentration must come up against this maximum, limiting the alteration in the balance of equations (1) and (2) and preventing a complete diuresis.

Hence, although there are reasons for believing that albumin infusions might tend to produce a diuresis in every case, there are also factors which alter the basic equilibrium constants and might prevent this result; the most important is probably the maximum rate of protein absorption by the already overloaded peritoneal lymphatics. Although final evidence of the factors governing the formation and elimination of ascitic fluid is still lacking, it seems that the above hypothesis fits the available facts better than anything previously suggested, and might serve as a basis for further investigation.

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## APPENDIX

*Patient III.* A man aged 65 was referred on 13.8.58 with a history of excessive intake of alcohol for many years, although his dietary history was good.

He had felt generally unwell for nine months, and more recently had developed ascites and oedema. The condition was temporarily controlled by the use of a high-protein diet, prednisone 15 mg. per day, and salt restriction and diuretic therapy, but he further deteriorated, and was readmitted on 1.11.58. Examination revealed marked wasting, no jaundice, but gross ascites and oedema, and after paracentesis the liver and spleen were found to be impalpable. There was no anaemia (haematocrit level 46 per cent.).

Albumin was infused at 50 g. per day to a total of 1,800 g., and a small natriuresis was observed, the maximal sodium excretion being 50 m-equiv. per day. The serum albumin reached a maximum of 4·5 g. per 100 ml., but there was no sign of reabsorption of the ascites, and the attempt was abandoned, apart from maintenance infusions to keep the serum level within the normal range. Ten days later a strangulation occurred of a small umbilical hernia, and this was successfully repaired surgically; but a further 10 days later, 24 hours after a maintenance infusion of 100 g., a major haematemesis occurred. There were further haematemeses during the next seven days, although no signs of portal-systemic encephalopathy developed; an emergency portacaval anastomosis was therefore performed. During the succeeding week the patient's condition progressively deteriorated, and he died in coma on 12.1.59.

*Patient VI.* A woman aged 33 was referred in October 1957 with ascites, which had proved intractable to diuretic therapy for three months. At the age of 19 she had developed pulmonary tuberculosis, which had rapidly resolved, and in addition she suffered from severe rheumatoid arthritis. There was no history of jaundice or excessive intake of alcohol, and the menstrual periods were normal. There was considerable rheumatoid deformity of the hands, elbows, hips, knees, and ankles. The skin was pigmented, and showed scratch marks; there were no spider naevi, 'liver' palms, or oedema, but considerable ascites. There was a normochromic, normocytic anaemia (haematocrit level 36 per cent.). Liver biopsy revealed an infiltration of the portal tracts with mononuclear cells and fibrous tissue, but without disorganization of the lobular architecture.

Daily infusion of 50 g. albumin resulted in a large increase in urinary sodium on the second day, and the ascites disappeared rapidly. The patient attended at weekly intervals for infusions for 12 months, and since then every two weeks; the serum albumin concentration is stable at 3·4 g. per 100 ml. In the first six months following the diuresis the prothrombin activity of the plasma increased from 40 to 90 per cent., where it has since remained. Treatment with cortisone acetate was commenced on 5.6.58, and has been continued to the present time at 50 mg. per day; there was an immediate fall in the serum bilirubin concentration to less than 1 mg. per 100 ml., and a slow increase in haemoglobin from 8·0 to 13·0 g. per 100 ml. Apart from one occasion when uterine bleeding resulted in a sudden drop, the haemoglobin concentration has remained between 11 and 13 g. per 100 ml. The patient remains in good general health, and is free from ascites and oedema.

*Patient VII.* A woman aged 65 was admitted to a surgical ward on 21.7.58 on account of abdominal pain. Laparotomy revealed cirrhosis of the liver and ascites. The ascites worsened after operation, and in spite of salt restriction and diuretics it was not possible to induce a satisfactory diuresis. There was no history of alcoholism or jaundice. On examination there was slight icterus, and moderate oedema, but no detectable ascites. The liver and spleen were impalpable; there were no signs of portal-systemic encephalopathy, no spider naevi, and no 'liver' palms. There was moderate anaemia (haematocrit level

38 per cent.), the serum complement was normal (1·3 units), and the urine contained a number of pus cells, but no protein. Albumin was infused at the rate of 50 g. per day, and there was a satisfactory diuresis of the oedema fluid, but ascites then appeared, and for a time continued to accumulate until the serum albumin reached 4·3 g. per 100 ml., when a final complete diuresis occurred. At the maximum serum albumin concentration (4·4 g. per 100 ml.), albuminuria occurred to the extent of 3 g. per day, but disappeared as the plasma concentration fell. The patient was discharged from hospital free from ascites and oedema, and feeling well. Infusions continued at weekly intervals, but abdominal pain developed and proved difficult to control. A small amount of ascites then returned, accompanied by severe malaise; the serum albumin level dropped to 3 g. per 10 ml. As the liver biopsy had shown a severe degree of inflammatory reaction, the patient was now treated with cortisone acetate 100 mg. per day, and the malaise diminished. Two weeks later, however, there was a severe haematemesis, and within an hour she was in deep hepatic coma, which lasted four days until her death on 6.12.58. Autopsy revealed a deep chronic gastric ulcer containing a large open blood-vessel. The kidneys were normal, the liver was grossly cirrhotic, and there was a small amount of ascites.

*Patient X.* A woman aged 25 was referred on 26.3.59, with the history that after a mild attack of jaundice in 1950 she had never been really well, and had suffered intermittent attacks of malaise, and slight jaundice. In 1957 she developed oedema of the ankles, and it was noted that the serum albumin concentration was 2·6 g. per 100 ml.; it subsequently fell to less than 1 g. per 100 ml. She was treated with diuretics and salt restriction, and the oedema disappeared, but she continued to feel unwell; with each intercurrent infection the oedema returned, and she was forced to relinquish her employment. Finally, after a severe attack of diarrhoea in January 1959, she lapsed into coma for seven days; on her recovery from this episode albumin therapy was commenced. On examination at this time she was found to be lethargic and miserable, with a marked 'flapping' tremor. There was slight foetor hepaticus, many spider naevi, and moderate ankle oedema, but no ascites. The liver and spleen were impalpable. Sputum grew a pyogenic staphylococcus, and a small amount of pleural fluid was found to contain many polymorphonuclear leucocytes. She was moderately anaemic (haematocrit level 39 per cent.).

Daily infusions of 50 g. albumin produced a rapid and complete diuresis of all oedema fluid, and restored the patient to normal well-being. During this period of daily infusions there was a marked exacerbation of acne vulgaris, which has since subsided. She was discharged free from oedema and ascites, and has remained in good health for a further 18 months, attending weekly for infusions of 50 g. albumin. She has resumed a full-time occupation, and says that she feels better than at any time during the past nine years. There have been no further attacks of portal-systemic encephalopathy.

## *Summary*

A study is reported of the treatment with intravenous albumin infusions of 13 patients with cirrhosis of the liver, complicated by ascites and oedema, which had proved resistant to conventional methods of treatment. In 10 patients it was possible to obtain a level of serum albumin of at least 4 g. per 100 ml., and of these 10 patients a complete diuresis was seen in seven. These seven patients were discharged from hospital capable of living a practically

normal life, and although still subject to other complications of the disease, which have resulted in the subsequent death of most of the patients studied, they were none the less maintained free from oedema and ascites for the remainder of their lives—that is, for periods of up to three years at the time of writing.

Changes in protein concentration in plasma and ascitic fluid have been closely studied, and it is observed that, although there is an increase in the rate of catabolism of albumin, there is also an apparent disappearance of more than half of the infused protein outside the 'exchangeable pool'; it is suggested that this protein probably moves into depleted intracellular tissues. Changes in concentration of globulins are readily explained by shifts to extravascular sites. On the basis of an analysis of the relative concentrations of the different proteins of plasma and ascitic fluid, a hypothesis is advanced of transcapillary selective diffusion and non-selective lymphatic reabsorption controlling the rate of passage of protein to and from the ascitic fluid.

Varying degrees of widening of the gap between the colloid osmotic pressure of the plasma and that of the ascitic fluid have been seen, and it is suggested that this widening corresponds to increasing portal pressure. In support of this view, the degree of widening has been found to be closely equated with the degree of expansion in blood volume which, it is argued, reflects portal pressure.

The mechanism of diuresis is examined, and it is suggested that the precipitating factor is expansion in blood volume, and that the continuing rise in colloid osmotic pressure results in the elimination of the oedema fluid. It is emphasized that complete reabsorption of ascites must result primarily from net removal of its contained protein. The reasons why this should occur in some patients and not in others are considered.

From these results there appears to be a group of patients with cirrhosis who stand to benefit greatly from albumin infusions. There is, as yet, insufficient indication for using the treatment as long as the patient can be kept free from retained fluid, and feeling well, by salt restriction and diuretics. When either of these two criteria is no longer satisfied, however, albumin therapy is indicated; it can benefit the majority of such patients and return at least 50 per cent. of them to an active life. Once a successful response has been obtained, it seems from the present series that the benefit persists, though only with maintenance infusions, which are probably required for the remainder of the patient's life.

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